ASSESSMENT OF APICAL SEALING ABILITY OF RETROGRADE FILLING MATERIALS WITH GIC, MTA, BIODENTINE AND BIOAGGREGATE: AN IN VITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH - IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

2015-2018

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"ASSESSMENT OF APICAL SEALING ABILITY OF RETROGRADE FILLING MATERIALS WITH GIC, MTA, BIODENTINE AND BIOAGGREGATE: AN IN VITRO STUDY"** is a bonafide work done by **Dr. VISHNUVARDHINI. S**, Post graduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **BRANCH-IV, CONSERVATIVE DENTISTRY AND ENDODONTICS,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2015-2018.

Date: 18-01-2018

Place: TIRUCHENGODE

Signature of the H.O.D

Dr. Vaiyapuri Ravi, M.D.S.,

Professor and Head of the Department,

Department Of Conservative Dentistry & Endodontics.

Signature of the Guide

Dr. A.S.Prasad, M.D.S.,

Reader and Guide,

Department Of Conservative Dentistry & Endodontics.

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT

AND HEAD OF THE INSTITUTION

This is to certify that **Dr. VISHNUVARDHINI. S**, Post graduate student (2015-2018) in the **DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS,** Vivekanandha Dental College for Women, has done this dissertation titled **"ASSESSMENT OF APICAL SEALING ABILITY OF RETROGRADE FILLING MATERIALS WITH GIC, MTA, BIODENTINE AND BIOAGGREGATE: AN IN VITRO STUDY"** under our guidance and supervision in partial fulfillment of the regulations laid down by the Tamilnadu Dr. M.G.R. Medical University, Chennai-600032 for **M.D.S (Branch-IV) CONSERVATIVE DENTISTRY AND ENDODONTICS.**

Seal & Signature of the H.O.D

Dr. Vaiyapuri Ravi, M.D.S.,

Professor and Head of the Department,

Department Of Conservative Dentistry&

Endodontics.

Dept. of Conservative Dentistry &
Endodontics
Vivekanandha Dental College for Women
Elayampalayam - 637205.
Tiruchengode-Tk Namakkal Dt. TamilNadu

N. Balan

Seal & Signature of the Principal

Dr. N.Balan, M.D.S.,

Principal,

Vivekanandha Dental College for Women,

Elayampalayam, Tiruchengode.

PRINCIPAL
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam - 637205.
Tiruchengode-Tk. NamakkalDt. TamilNadu

DECLARATION

TITLE OF DISSERTATION	**ASSESSMENT OF APICAL SEALING ABILITY OF RETROGRADE FILLING MATERIALS WITH GIC, MTA, BIODENTINE AND BIOAGGREGATE: AN IN VITRO STUDY**
PLACE OF STUDY	**VIVEKANANDHA DENTAL COLLEGE FOR WOMEN, TIRUCHENGODE**
DURATION OF THE COURSE	**3 YEARS (2015-2018)**
NAME OF THE GUIDE	**Dr. A.S.PRASAD, M.D.S.,**
HEAD OF THE DEPARTMENT	**Dr. VAIYAPURI RAVI, M.D.S.,**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode. In addition, I declare that no part of this work will be published either in print or electronic without the permission of the Guide who has been actively involved in the dissertation. The author has the right to reserve publishing of work solely with prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode.

Head of the Department | Guide | Signature of the Candidate

CERTIFICATE – II

This is to certify that this dissertation work titled **"ASSESSMENT OF APICAL SEALING ABILITY OF RETROGRADE FILLING MATERIALS WITH GIC, MTA, BIODENTINE AND BIOAGGREGATE: AN IN VITRO STUDY"** of the candidate **Dr. VISHNUVARDHINI. S,** with registration Number **241517553** for the award of **MASTER OF DENTAL SURGERY** in the branch of **CONSERVATIVE DENTISTRY AND ENDODONTICS.** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **2** percentage of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

Dept. of Conservative Dentistry &
Endodontics
Vivekanandha Dental College for Women
Elayampalayam - 637205.
Tiruchengode-Tk Namakkal Dt. TamilNadu

URKUND

Urkund Analysis Result

Analysed Document:	DISSERTATION FINAL.docx (D34382748)
Submitted:	1/5/2018 3:35:00 PM
Submitted By:	s.vishnuvardhini@gmail.com
Significance:	2 %

Sources included in the report:

http://www.ijcdmr.com/index.php/ijcdmr/article/viewFile/124/103
http://www.jcd.org.in/article.asp?
issn=0972-0707;year=2013;volume=16;issue=6;spage=573;epage=575;aulast=Pawar
http://intjdc.org/index.php/intjdc/article/download/73051171/pdf_228

Instances where selected sources appear:

10

ACKNOWLEDGEMENT

First of all, I thank GOD ALMIGHTY, from whom I receive everything for accomplishing my desires.

My sincere thanks and deep sense of gratitude to **Dr. Capt.S.Gokulanathan**, **B.Sc**, **M.D.S., (Dean) and Dr. N.Balan, M.D.S., (Principal),** Vivekanandha Dental College for Women, for permitting me to pursue this work.

With submissive ambition, I aspire to register my gratitude to my respected Mentor and diligent Professor and Head of the Department**, Dr. Vaiyapuri Ravi, M.D.S.,** Department of Conservative Dentistry and Endodontics, Vivekanandha Dental College for Women, for his innovative ideas, invaluable counsel and immeasurable encouragement throughout the course of the work. His care, matchless theoretical and clinical skills, coupled with ideals and unwavering guidance and constant support during my postgraduate tenure has enabled me to successfully conclude this effort. This work would not have seen the light of the day without his affectionate and compassionate counseling, which reposed the confidence in myself to undertake the challenges in the work.

I am greatly indebted to my Guide **Dr. A.S.Prasad, M.D.S.,** Reader, Department of Conservative Dentistry and Endodontics, Vivekanandha Dental College for Women for his knowledge and experience in guiding me to understand the concept behind this research work. His valuable criticism, constructive suggestions and constant support that enabled me to comprehend this dissertation and reach its successful culmination.

I sincerely thank **Dr. A.AndamuthuSivakumar, M.D.S.,** Professor, who has always been a source of support and encouragement at any moment. I am grateful to him for sparing his valuable time in guiding me throughout this work. His profound knowledge and valuable suggestions had benefited me in every facet of my academic life.

I would like to extend my heartfelt gratitude to **Dr. J.S.Sivakumar, M.D.S.,** Reader, for his invaluable guidance and support at every juncture throughout my postgraduate curriculum.

I take this opportunity to express my sincere heartfelt thanks to **Dr. P.V.Syamala, M.D.S,** Professor, **Dr. Aji Markose, M.D.S.,** Professor**,** **Dr. J.Saravana Priyan Soundappan, M.D.S.,** Reader, **Dr. M.Chittrarasu, M.D.S.,** Senior Lecturer and **Dr. Divya.V, M.D.S.,** Senior Lecturer for rendering me their valuable suggestions and generous support during this course of work.

I am extremely thankful to **Dr. Maheshwaran.T, M.D.S.,** Reader and my friend **Dr. Keerthi Priyadharshini.T,** postgraduate student, Department of Oral Pathology and Oral Microbiology, Vivekanandha Dental College for Women, for their sincere efforts and constant help during stereomicroscopic analysis of tooth samples.

I also sincerely thank **Mr. Tullanithi.K.M,** Reader, Department of Biochemistry, Vivekanandha Dental College for Women, for helping me in the preparation of dye solution.

I am thankful to **Mr. Nantha Kumar, M.Sc., M.Phil., Ph.D., M.B.A., PG DSBS.,** for his guidance in the statistical works of this study.

I also wish to acknowledge **Dr. Dhinakaran.L.M, M.D.S.,** Endodontist, Coimbatore, who has been a continous source of inspiration to me and helped me in this little journey with his unwavering support and constant encouragement. His natural flair and passion towards the subject, not only gave me a solid foundation in Endodontics but also inculcated the need to combine knowledge with ethical practices in me.

I am grateful to my batch mates **Dr. Iswariya.R, Dr. Karthipriya.G** for their kind help and support during this period of study. It would not be justifiable on my part if I do not acknowledge the help of my juniors **Dr. Chandrika.R.P, Dr. Sowmiya.T, Dr. Vineetha.C.S, Dr. Anuradha.R.S, Dr. Brindha.L** and **Dr. Pushpalatha.K** for their continued support and encouragement throughout my postgraduate programme.

Words are not enough to express my sincere love and gratefulness to my father **Mr. S.Sakthivel** and mother **Mrs. S.Kalaivani,** without whom this academic session would just have been a dream. They were with me in all ups and downs of my life.

I also like to extend my heartfelt gratitude to my grand-father **Mr. K.P.Sargunam,** grand-mother **Mrs. S.Pushpavalli**, uncle **Mr. C.Manoharan** and aunt **Mrs. S.Lathamani**.

They all made possible my tomorrow by sacrificing their yesterday and I once again thank them for their evergreen love and support for which I will be eternally indebted.

CONTENTS

INTRODUCTION

INTRODUCTION

The main goal of endodontic treatment is the correct diagnosis, optimal mechanical and chemical preparation of the root canal space and creating a hermetic seal that prevents all pathways of communication between the canal space and periradicular tissues.[1] Although endodontic treatment has a high success rate, failures do occur. This can be attributed to various factors such as variations in the anatomy of the teeth, persistence of bacteria (both intracanal and extracanal), inadequate filling of the canals, overextensions of the root filling materials, improper coronal seal, untreated canals (both major and accessory), iatrogenic procedural errors like poor access cavity design, and complications that occurs during instrumentation such as ledges, perforations or separated instruments.[2]

In cases where non-surgical endodontic treatment proves unsuccessful or are contraindicated, surgical endodontic therapy is needed to save the tooth. Root-end resection is the most common surgical procedure followed in periradicular surgery. The periapical surgery procedure involves access to the affected area, root-end resection, root-end preparation, periradicular curettage and placement of a suitable root-end filling material.[3]

The root-end filling is necessary to provide adequate apical seal, preventing the egress of micro-organisms from the root canal system into the periradicular tissues. The ideal root-end filling material should be: non-toxic, non-carcinogenic, non-corrosive, non-staining to periapical tissues, biocompatible with host tissues, able to stimulate the regeneration of the periodontium, insoluble, dimensionally stable, unaffected by moisture, adherent to dentine, radiopaque, easy to use and have a long shelf life.

Various materials have been suggested and tested in the quest to fulfil all these ideal requirements. Amongst those proposed are: amalgam, gutta percha, Cavit (3M ESPE, St Paul, Minnesota, USA), glass ionomer cement, IRM (Dentsply/Maillefer, Ballaigues, Switzerland), Super EBA, (Harry J Bosworth Co. Skokie, Illinois, USA), composite resin, compomer, gold foil, Diaket (3M/ESPE, Seefeld, Germany), polycarboxylate cement, Mineral trioxide Aggregate (MTA), casteroil polymer, Ceramicrete, Endosequence, etc.,[4]

Dental amalgam was first used as retrograde filling material by **Farrar** in **1884** and has since been the most widely used material. The advantages of amalgam are that it is inexpensive, readily available, radiopaque and insoluble in fluids. The disadvantages includes initial microleakage, electrochemical corrosion, induction of inflammation of adjacent periradicular tissues, amalgam tattoo formation, the need for an undercut in cavity preparation, zinc toxicity, delayed expansion and concerns over the introduction of mercury into periradicular areas.[5]

Gutta percha derived from the sap trees mostly of the Palaquium gutta. It was introduced by **Bowman 1867** to fill the root canal space and composed of 20% gutta percha as matrix, 66% zinc oxide as a filler, 11% heavy metal sulphates as radiopacifiers and 3% waxes or resins as plasticizer. Both heat sealed and thermoplastic gutta percha should be used with an endodontic sealer to aid in sealing as they lack any molecular binding with root dentin. Cold burnishing of gutta percha at the time of root end resection has been proposed technique for sealing the root-end, however evidence shows that this results in significantly more leakage than amalgam and IRM. So, the use of gutta percha as a root end filling cannot be advocated due to its poor sealing ability.[6]

Gold foil as root-end filling material was reported by **Schuster** in **1913 and Lyon** in **1920**. It exhibits excellent marginal adaptation and biocompatibility.[7] But the drawback of use of gold as retrograde filling materials is that a moisture free environment is required for the placement of gold and technique sensitive.

Cavit (3M ESPE) is a calcium sulphate based temporary restorative material which is available in a premixed state that is simple to manipulate and apply to the root-end cavity. It is a hygroscopic material that undergoes linear expansion and sets when mixed with water, resulting in a good marginal adaptation, provided a minimum thickness of 3.5mm of Cavit is placed.[8] However, they are soluble and disintegrates when contacts with tissue fluids and therefore cannot be used as a root-end filling.

In **1962, Nichols** mentioned that zinc oxide eugenol cements can be used as retrograde filling materials because of its good handling properties and satisfactory post-operative results. However, they are weak, soluble and had a long setting time. Two modifications of zinc oxide eugenol cements have been recommended as root-end filling materials:

a) IRM – it contains 20% polymethyl methacrylate added to the zinc oxide powder and the eugenol liquid remains the same.
b) Super EBA – contains 60% zinc oxide, 34% silicon dioxide and 6% natural resin as powder component and liquid composed of orthoethoxy benzoic acid (EBA) & 37.5% eugenol.[9]

Though these reinforced zinc oxide eugenol cements resists dissolution and provides better sealing than amalgam & gutta percha, they exhibit cytotoxicity due to release of eugenol from the set mass thereby limiting its usage as root-end filling material.[10]

Glass ionomers cements were introduced in 1970s which are based on the reaction of ion-leachable, acid soluble calcium fluoro aluminosilicate glass particles with polyalkenoic acid and possess adhesive properties by forming a chemical bond with dentin. They induce an intense inflammatory response which resolves and is replaced by bone.[10] Resin modified glass ionomer cement which was first described by **Antonucci et al**, can be used as a potential retrograde filling material as it possess improved handling properties and good adaptation & sealing ability which was significantly better when compared to that of amalgam. But maintenance of dry field during the placement still presents a challenge as they are very sensitive to moisture contamination which may interfere with the dentin bond.[11]

Composite resins along with dentin bonding agent can be used as retrograde filling material. **Rud et al** have shown excellent long term clinical success with Retroplast composite resin root-end fill and Gluma dentin bonding agent.[12] Periapical biopsies of teeth with composite resin retrograde fillings have shown deposition of cementum and reformation of periodontal ligament over the resin fillings. This is because of high amounts of EMD (enamel matrix derivatives) were found to adhere to the composite resin which helps to promote periodontal regeneration.[13] But the limitations of use of composite resin as root-end filling materials are they are technique sensitive and maintenance of a completely dry field during placement is essential.

Compomers are polyacid modified composite resins developed to combine the fluoride releasing property of glass ionomer cements with mechanical properties of composite resin. The monomer contains acidic functional groups and the material sets via a free radical polymerization reaction. It does not bond to the tooth structure like glass ionomer cement but need a bonding agent like composite resins. In the tooth where compomer is placed as retrograde filling material, the gingival tissues appear to adhere to the material allowing fibroblasts to reform around the root apex. Dyract is one of the compomer mostly used as root-end fillings shows good anti-bacterial effects against *P.gingivalis*, *P.intermedia*, *P.endodontalis* and *F.nucleatum* due release of residual monomers and additives after polymerization.[14] The main disadvantage of compomer are their low biocompatibility resulting in inflammation and limited bone formation.

Diaket is a polyvinyl resin that is formed between zinc oxide and diaketone is normally used as a root canal sealer. It has been used as a root-end filling material when mixed in thicker consistency. It has good radiopacity and a working time of more than thirty minutes as a root-end material, diaket is shown to have superior sealing qualities when compared to amalgam, good healing response characterized by bone apposition, reformation of periodontal ligament and deposition of new cementum.[15]

Ceramicrete is an inorganic phosphate ceramic binder material that sets by acid-base reaction to form potassium magnesium phosphate hexa-hydrate ceramic matrix phase. The composition of ceramicrete dental material includes hydroxyapatite powder and cerium oxide as radiopaque fillers. They exhibit sealing property, biocompatibility and radiopacity when used as root-end filling material.[16]

Castor oil polymer (COP), a relatively new material has demonstrated a good potential as root-end filling material. This material possess high biocompatibility, good sealing properties and easy to handle. This biopolymer is composed of chains of fatty acids whose molecular structures are similar to that of lipids present in human body. In a study conducted by Giovanna et al in 2009, COP produced excellent sealing ability as retrograde filling materials when compared to that of MTA and glass ionomer cements.[17]

In 1993, Mineral trioxide Aggregate (MTA) was developed by **Torabinejad** & his co-workers at Lomba Linda University, California. MTA has shown to produce excellent seal and hard tissue repair compared with other root-end filling materials. The components of MTA includes tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and bismuth oxide (for radiopacity).[18] Hydration of the powder forms a colloidal gel that hardens. According to a study done by **Pitt Ford and Chong in 2003**[19] comparing MTA & IRM, the use of MTA showed a higher success rate as retrograde material. The main advantages of MTA are its biocompatibility, bioactivity, anti-bacterial properties, good sealing properties and potential to stimulate cementogenesis. The disadvantage of it is slow setting and less resistance against washing out during placement.[20]

Biodentine introduced in 2010 is a calcium silicate based material was recently used as root-end filling material. It is also used for perforation repair, apexifications, resorption repair, etc. The main component is highly purified tricalcium silicate powder that contains small amounts of dicalcium silicate, calcium carbonate and a radio-opaquer.[21] It has increased physico-chemical properties like short setting time, high mechanical strength which makes it clinically easy to handle and biocompatible.[22] The interfacial properties of dentine-Biodentine interface were studied under microscope and tag-like microstructures were detected. Moreover, the flowable consistency of Biodentine aids in dentinal tubule penetration and provides better sealing properties to the material.[23]

Bioaggregate is a new bioceramic material marketed as iRoot BP plus (BP-RRM; Innovative BioCeramix Inc, Vancouver, BC, Canada). It has been indicated for use in root-end filling material and for root resorption repair. It is a ready-to-use premixed bioceramic paste which contains tricalcium silicate, dicalcium silicate, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic and filler agents. It sets & hardens in the presence of water and it requires a minimum of 2 hours to set.[24] In a study done to compare the cytotoxicity of ProRoot MTA and Bioaggregate, it showed a significantly better inflammatory reaction and foreign body reaction than the MTA group which indicates Bioaggregate is more biocompatible than MTA.[25] An in vitro study done by **Oncel Torun et al in 2015**[26] confirmed that iRoot BP Plus Bioaggregate putty material facilitated odontoblastic differentiation and is compared to that of white MTA.

The quality of apical seal achieved by root-end filling materials has been assessed by various methods such as degree of dye penetration, radioisotope penetration, bacterial penetration, electrochemical means and fluid filtration techniques. In this, the most popularly used method is dye penetration method and it is accepted as a valid method for the initial evaluation of experimental retrograde filling materials.[27] Various dyes that can be used to assess the sealing ability includes India ink, basic fuchsin, silver nitrate with developer and methylene blue. The most widely used dye is methylene blue but the main disadvantage is when it comes in contact with alkaline materials, it becomes colourless and loses its marking ability.[28] So, rhodamine B dye which is not affected by alkaline material is used as an alternative in our study to assess the microleakage.

AIM AND OBJECTIVE

AIM AND OBJECTIVE OF THE STUDY

The aim and objective of the study is to compare and evaluate the apical sealing ability of four different retrograde filling materials by dye penetration method using stereomicroscope.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

J.Danin et al (1992)[29] done a quantitative radioactive analysis of microleakage with four different retrograde materials such as Amalgam, Glass ionomer cement, Sealapex and Composite resin. The study involved a time period of 1 year by placing the paperpoint impregnated with radioactive solution in the prepared root canals of the retrofilled samples and the measurements were made at regular intervals. The results of the study showed calcium hydroxide based sealer Sealapex and light cured Composite resin showed less apical leakage than Amalgam and Glass ionomer cement. The authors suggested that less apical leakage seen with Sealapex may be due to cementum deposition by releasing large amounts of calcium hydroxide which enhances apical closure.

F. Ozata et al (1993)[30] done an in vitro study to compare the sealing ability of high-copper amalgam, glass ionomer cement & silver glass ionomer cement as retrofilling materials when used with and without varnish using dye penetration method and analysed under a stereomicroscope. The results of the study indicated that conventional glass ionomer cement with varnish had significantly less dye leakage than high-copper amalgam. The author revealed that this can be due to the reason that use of varnish over the glass ionomer cement is important in preventing the dehydration of the cement during the setting reaction and to protect the material from moisture contamination leading to improved seal of the glass ionomer cement. This finding was in agreement with the study done by **Schwartz & Alexander (1988) and Barkhorda et al (1989).** Also this study demonstrated that surface treatment with a dentine conditioner in the cavity preparation to remove smear layer is recommended to enhance adhesion of glass ionomer cement.

Mahmoud Torabinejad et al (1994)[31] compared the amount of dye leakage in the presence versus absence of blood in root-end cavities filled with amalgam, Super EBA, IRM and MTA. The results of this study concluded that presence or absence of blood had no significant effect on the amount of dye leakage and mean dye leakage was significantly less with MTA than other tested materials. The author revealed that the existence of the dye leakage were due to the reason that interface between the root end filling materials and the dentinal walls was uneven which indicates the presence of potential gap for bacterial penetration.

Peter A. Gilheany et al (1994)[32] designed a study to evaluate the apical leakage associated with various depth of retrograde filling placed in root apices which had been resected at one of the three different angles and the leakage was assessed with hydraulic cconductance apparatus. This study showed that increasing the depth of the retrograde filling significantly decreased apical leakage and that there was significant increase in leakage as the amount of bevel increased, due to leakage through the resected apical dentin. The optimum depths for a retrograde cavity are 1.0, 2.1 & 2.5 mm for 0^0, 30^0 and 45^0 angle of resection respectively. Apical leakage can be minimized by resecting the root apex at an angle of 0^0 to the long axis. Finally, the author concluded that, the permeability of the resected apical dentin & microleakage around the retrograde filling material both have a significant influence on apical seal.

Mahmoud Torabinejad et al (1995)[33] conducted a study to determine the chemical composition, pH & radiopacity of MTA and also compared the setting time, compressive strength and solubility of MTA with Amalgam, Super EBA and Intermediate Restorative Material (IRM). The results of this study showed that main molecules present in MTA are calcium and phosphorous ions. The pH of MTA was initially 10.2 and after mixing it rises to 12.5 in 3 hours. MTA is more radiopaque than

Super EBA and IRM. Amalgam had the shortest setting time (4min) and MTA the longest (2hrs 45min). At 24 hours, MTA had the lowest compressive strength (40MPa) among the materials, but it increased after 21 days to 67MPa. Among the tested materials, only IRM showed solubility and no other material showed solubility.

Massimo Gagliani et al (1998)[34] designed a study to evaluate how the apical root resection angle and cavity made by ultrasonic retrotips may influence the apical seal using dye penetration method under a stereomicroscope. The results of the study showed that there was less infiltration both in dentin and in the space between the filling & dentinal wall in the group with 90^{o} angle when compared to group with 45^{o} angle. None of the specimens presented with degree of dentinal infiltration as long or longer than the depth of 3mm preparation. This indicates that retreating to this depth of 3mm provides a good apical seal, regardless of whether the resection angle is 90^{0} or 45^{0}.

J.Aqrabawi et al (2000)[35] assessed the effectiveness of MTA in providing an apical seal in comparison with Amalgam & Super EBA cement by using dye penetration method. The outcome of the study showed that MTA had less dye leakage compared with Amalgam and Super EBA cement as it provides hermetic seal with the root dentine. The author also revealed that when a filling material does not allow penetration of small molecules, it has the potential to prevent leakage of substances such as bacteria and their by-products.

Beatris Farias Vogt et al (2006)[36] evaluated the dentin penetrability of three dye (rhodamine B, Silver nitrate & Methylene blue) in root-end cavities filled with MTA. This test showed that the dyes presented with different degrees of penetration into apical dentin. The lowest leakage results were observed in silver nitrate group, intermediate results were seen in Methylene Blue group and highest penetration were observed in rhodamine B group. This is because that silver nitrate produces some kind of chemical reactions with the retrofill material and MTA provokes 73% reduction in optical density of methylene blue leading to false results in microleakage studies. So, these both dyes should be avoided when using MTA as retrofill material. The author suggested that the most appropriate tracer solution to evaluate the sealing capacity of MTA is rhodamine B dye. Also the findings of the study demonstrated that selection of tracer solution is very important as it could influence the results of microleakage tests.

Marcia Carnciro et al (2006)[37] investigated the apical leakage of retrograde cavities filled with Portland cement, ProRoot MTA and Sealapex with addition of zinc oxide using dye penetration method. The results of the study revealed that there was no statistically significant difference in the mean microleakage values and hence all three tested materials presented with similar marginal sealing ability. This result was in accordance with **Holland et al (2001)** who observed a very similar behaviour between Portland cement and MTA in inducing mineralised tissue deposition, as they both have same properties. In the present study, Sealapex plus zinc oxide also presented with similar leakage as the other two cements because it also allowed larger mineralised tissue deposition at the periapical level.

L.K.Post et al (2010)[38] investigated the effect of different apicoectomy angles, instruments used in root-end preparation and dental materials used in retrofilling by an in vitro study. Root ends of 80 single rooted teeth were resected at 45 or 90 degrees. For each type of apicoectomy, root-end cavities were prepared with either a round carbide #2 bur or an S12/90D ultrasonic tip. The root-end cavities in each sub group were filled with silver amalgam or MTA (Angelus) and the specimens were immersed in 0.2% rhodamine B dye for 24hrs. Sealing was evaluated based on the dye cross sectioned dentin area. The study revealed that the angle of apicoectomy and the type of root-end preparation did not affect the degree of dye penetration. The only significant factor affecting microleakage was the dental material used for retrofilling with MTA exhibiting less leakage.

Amany. E. Badr et al (2010)[39] demonstrated the marginal adaptation and cytotoxic effect of PMMA bone cement, MTA & amalgam as retrograde filling materials. The data obtained from the study revealed that both bone cement & MTA exhibited better adaptation to the dentinal walls and they both showed lesser cytotoxicity than amalgam. The proper adaptation of bone cement was due to its maximum increase in the volume of cement during polymerisation before shrinking as explained by **Charney et al (1970)** and the good adaptation of MTA to cavity margins might be intrinsically linked to the nature of the material.

Shahriar Shahi et al (2011)[40] compared the sealing ability of White MTA, Gray MTA, White Portland cement and Gray Portland cement as root-end filling materials by dye leakage test using stereomicroscope at 16x magnification. The results of the study demonstrated no statistically significant difference among the studied groups. This can be due to that the components of MTA and Portland cements are similar, these materials are expected to have similar properties and effects. The author suggested that Portland cement being cheaper and has apparently same sealing ability as MTA, Portland cement could be considered as a possible substitute for MTA as root-end filling material.

Shokouhinejad N et al (2012)[41] evaluated the bioactivity of Bioaggregate material by immersing them in Phosphate buffered solution (PBS) for 2 months period. Scanning electron microscopic analysis was carried out to find the precipitation of apatite crystals on the surface of the cement and / or at the dentin-cement interface and they were analysed elementally by energy dispersive X-ray instrument. Their results confirmed that Bioaggregate are bioactive and its bioactivity became larger with increase in time period.

G. De-Deus et al (2012)[42] verified the cytocompatability of iRoot BP Plus (Bioaggregate) and compared it with white ProRoot MTA. In this in vitro study, thirty six extracted human maxillary incissors were taken and root canals were prepared and obturated. Then apical 3mm root end resection perpendicular to the long axis of the tooth was carried out and retrograde preparations were done. They were then restored with WMTA & iRoot BP Plus (Bioaggregate) and was exposed to culture media containing Human osteoblasts cells extract for 24 hours. A multiparametric cell viability assay was performed evaluating mitochondrial activity, membrane integrity and cell density. The results of this study showed both iRoot BP Plus & White MTA were biocompatible and no critical cytotoxic effects were induced by them.

Amin Salem Milani et al (2012)[43] designed an in vitro study in which they compared the sealing ability of resected roots filled with Mineral Trioxide Aggregate (MTA) and Calcium Enriched Mixture (CEM) cement. The methodology includes seventy maxillary anterior teeth in which root canals were prepared and they were randomly divided into four experimental groups (n=15) and two control groups (n=5). In Group 1 & 2, CEM & MTA was placed into the apical 6mm of the canals respectively. The remaining portion of the canals were filled with gutta-percha/AH26 sealer and finally 3mm of the root-ends were resected. In Group 3 & 4, the canals were first obturated with gutta percha/AH26. After which the root-ends were resected, retrograde cavities were prepared and filled with CEM & MTA in respective samples. Then all the samples were placed in India ink and maximum dye penetration was measured with a stereomicroscope. The results of the study showed that the resected orthograde materials presented with more dye leakage than the retrofilled materials which was statistically significant in case of CEM cement and CEM cement showed less microleakage compared with MTA in the resected or retrofilled state; however the differences were not statistically significant. Therefore this study revealed that if limited access prohibits retrofill placement, MTA or CEM can be used to fill the canal prior to root-end resection; as they have similar sealing ability.

El Sayed et al (2012)[44] compared the sealing ability of Diadent Bioaggregate, IRM, Amalgam and WMTA by an in vitro study when used as retrograde filling materials. In this study, they included sixty extracted human maxillary incissors which were sectioned at CEJ, instrumented and obturated with gutta-percha & resin sealer. Then they were randomly divided into two control groups and four experimental groups containing ten samples each. Dye penetration technique using 2% methylene blue dye solution was done to assess the apical leakage. The results of this study showed that high sealing ability was seen with Diadent Bioaggregate group.

Sabari Girish et al (2013)[45] done an in vitro study to assess the sealing ability of MTA, Polymethylmethacrylate (PMMA) bone cement and CHITRA Calcium phosphate cement (CPC) when used as root-end filling material using rhodamine B dye evaluated under a confocal laser scanning microscope and also to compare the seal of root-ends prepared using an ultrasonic retrotip & Er.YAG laser. The study results showed that PMMA bone cement is a better root-end filling material to prevent microleakage. MTA still continues to be gold standard root-end material showing minimum microleakage. The amount of dye penetration was found to be lesser in root-ends prepared with Er:YAG laser. This may be due to better preservation of the integrity of root-end cavities from the stand point of dentinal chipping. But the difference between the laser preparation and ultrasonic preparation was found to be not statistically significant.

Young- Eun Jang et al (2014)[46] done a study to evaluate the cytotoxicity, setting time and compressive strength of MTA, Biodentine & Bioaggregate. Cytotoxicity of these materials were evaluated using 2,3 bis (2 methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide XTT assay. Setting time and compressive strengths were performed following ISO requirements. The results of this study revealed that both Biodentine & Bioaggregate were biocompatible. Bioaggregate showed comparable cytotoxicity to MTA but inferior physical properties. Biodentine showed somewhat higher cytotoxicity but superior physical properties than MTA.

Shajak Pathak et al (2015)[47] conducted an in vitro study to compare and evaluate the sealing ability of four different root end filling materials such as GIC, IRM, MTA and Biodentine using scanning electron microscope. They stated that Biodentine exhibits better sealing among the tested four materials. This can be attributed to formation of tag like structures composed of calcium or phosphate rich crystalline deposits which increases over time hence minimizing the gap between the tooth and Biodentine.

Pankaj Kumar et al (2015)[48] investigated whether different manipulation methods of Biodentine influences its sealing ability when used as root end filling material. After root end preparation of totally 60 teeth samples, half of them were restored with Biodentine manipulated by machine trituration and remaining by manually mixed Biodentine. Dye penetration method was carried out and the samples were analysed under a stereomicroscope. It was seen that samples restored with Biodentine which was manually manipulated shows more microleakage whereas the material manipulated with machine trituration produced better sealing with less microleakage.

Prasanti Kumari Pradhan et al (2015)[49] investigated the study to evaluate the sealing ability of five different root-end filling materials like GIC, Super EBA, white MTA, gray MTA and Biodentine by dye penetration method. The overall results of the study showed that both forms of MTA & Biodentine provides a better seal than GIC & Super EBA. In the present study GIC & EBA showed more leakage inspite of their good dentin adhesive property. This may be attributed to their disadvantage of the materials such as moisture sensitivity, partial solubility in oral fluids and technique sensitivity.

Fatemah et al (2015)[50] done an in vitro study to compare the marginal adaptation of Cold Ceramic and MTA when used as retrograde filling material using SEM. This study included twenty extracted human single rooted teeth which were decoronated at CEJ & were instrumented using step-back technique. The root canals were obturated and root end resection of 3mm above the apex was carried out at 90 degree to the long axis of the tooth. Then the root end preparation was done and they were divided into two groups containing ten samples each. The two groups were retrofilled with Cold Ceramic and MTA respectively. The roots of these samples were cut horizontally from 1mm above the apical part and dentin-filling material interface was assessed by Scanning Electron Microscope. The results of this study showed that both of these materials had similar marginal adaptation.

Mayuri Mohan Naik et al(2015)[51] done an in vitro study to investigate the apical seal obtained after irrigation of root-end cavity with MTAD followed by subsequent retrofilling with MTA & Biodentine using dye extraction method with UV spectrophotometer. Irrigation regimen with MTAD improved the apical seal of Biodentine but decreased the apical seal of MTA. This can be attributed to removal of loosely attached smear layer opens up the dentinal tubules and creates a retentive surface for interlocking of the Biodentine molecules whereas the presence of citric acid in MTAD inhibits the penetration of MTA into the dentine.

Pragna Mandana et al (2015)[52] designed an in vitro study to evaluate the apical microleakage of root-end cavities filled with white MTA, Biodentine and light cure Glass ionomer cement using two different cavity preparation techniques such as conventional bur preparation and ultrasonic tip preparation. The results this study revealed that white MTA produced less microleakage when compared to Biodentine & light cure Glass ioomer and there is no statistical difference between the ultrasonic retrotip preparation & conventional bur preparation still ultrasonic producing less microleakage. The reason for white MTA showed better sealing may be due to its smaller particle size which means it has greater specific surface area that in turn causes an increase in wetting volume, water-binding capacity and hydration rate. As MTA is hydrophilic in nature, it undergoes setting expansion when it is cured in moist environment and thus the presence of moisture in the surgical field does not affect its setting or the properties. On the basis of the study, the author suggested that MTA with ultrasonic preparation is better root-end filling material to prevent microleakage.

Ankita Khandelwal et al (2015)[53] compared the sealing ability of Mineral Trioxide Aggregate and Biodentine as root-end filling material, and also the effect of different retro preparation techniques ie. conventional bur v/s ultrasonic tips on sealability of both the rot-end filling materials. The results of the study presented with highest mean microleakge with Mineral Trioxide Aggregate when compared with Biodentine and mean microleakage is minimum in Biodentine with ultrasonic preparation followed by Biodentine with bur preparation. In this study, sealing ability of both the filling materials is influenced by the root-end preparation technique. In all samples, root-end preparation with ultrasonic showed less leakage values. This can be attributed to the condition of the cavity surface left after the preparation technique. Cavities prepared with rotary burs are left with a greater amount of debris and smear layer in comparison to those prepared with diamond coated ultrasonic retrotips which prevents complete contact between filling material and cavity walls. Also this study showed that irrespective of preparation technique used, Biodentine still showed better sealing than MTA due to its smaller particle size of the Biodentine making it well adapt to cavity surface.

Jun Tian et al (2015)[54] investigated the effect of Bioaggregate on osteoclast differentiation, fusion and bone resorption by an in vitro study. This study revealed that Bioaggregate inhibits osteoclast differentiation, fusion and bone resorption. In addition they also provides insight about the mechanism by which calcium silicate based bioceramics inhibit osteoclastogenesis and bone resorption. Bioaggregate releases Si ions and small amounts of Sr ions which provides alkalinity which decreases the migration ability & fusion of RAW264 cells that are responsible for osteoclastogenesis. They also decreases the expression of RANK, TRAF6, NF-Kb & NFATc1 factors which are responsible for bone resorption. Hence in this study, they stated that Bioaggregate is a very useful material and because of these advantages it can be used for several clinical situations.

Dilek Helvacioglu–Yigit et al (2016) [55] conducted a study to evaluate the artifacts generated by four different root end filling materials using cone beam computed tomography. Twenty central incissors teeth were used in this in vitro study which were instrumented and obturated. Then the root end cavity was made in each sample and they were randomly divided into 4 groups containing five samples each which were retro filled with Amalgam, Super EBA, Biodentine and MTA. They were then placed in a skull with soft tissue simulation and scanned by using the Planmeca

Promax with different kVp: 66, 76, 84 & 96 with and without use of Metal Artifact Reduction (MAR) algorithm and with low, normal & high resolution and high definition. The Dose area product was calculated. The results of this study concluded that Biodentine, MTA, Super EBA generated few artifacts when used as root end filling compared to amalgam and use of 96 kVp with MAR & low resolution also reduced the artifacts.

Anurag Jain et al (2016) [56] conducted a study to compare the sealing ability of root-end filling material such as MTA, Portland cement, IRM & RMGIC in teeth with root apices resected at 0^0 and 45^0 angle using dye penetration method under fluorescent microscope. The results of the study showed that the root apex sealing ability of MTA was superior to other tested materials. This is because that MTA induces hard tissue barrier which would minimize the interaction between material & host tissues and produces better results where the issue of microleakage is concerned. Also the present study concluded that the root-end resection angulation whether 0^0 and 45^0 angle did not affect the sealing ability of all four materials. From this, the author suggested that in cases where clinically resecting the apex at 0^0 were difficult & might require more bone removal, placing the angle 45^0 can be used as an alternate which might help in achieving the same result as that of with 0^0 angle & with lesser removal of bone.

Harshit Srivastava et al (2016)[57] assessed the sealing ability of Glass ionomer cement, Biodentine, Mineral Trioxide Aggregate and Bone cement when used as retrograde filling materials using dye penetration method. The study revealed that Biodentine has better sealing property than the other three tested materials. This is because of the formation of tag-like structures of Biodentine when it comes in contact with root dentine and it has also less porosity & pore volume. Even Bone cement showed comparative sealing with root dentine which may be due to its excellent interlocking of cement with hard tissues.

Al-Hashimi et al (2016) [58] conducted a study to evaluate and compare the apical microleakage around retrograde cavities prepared with ultrasonic technique and filled with Biodentine. The results of this study showed significantly less microleakage in groups prepared with ultrasonic than conventional method. The author revealed that the reason for less microleakage observed in cavities preparation with ultrasonics is due to the geometry of the retrotip design which does not require a bevelled root-end resection, thus decreasing the number of exposed dentinal tubules and improving the sealing of retrofillings. Also the present study revealed less microleakage was seen with ultrasonic compaction of Biodentine when compared to conventional method of compaction. This is an agreement with Roberta et al who said that ultrasonic vibration made a higher performance of the condenser during the compaction procedure because it helped in better distribution & density of the material inside the retrograde cavity improving the flow, setting & sealing of the material to root end dentinal walls with fewer voids.

Teena Dsouza et al (2016)[59] evaluated the root-end sealing ability of white MTA combined with either distilled water, 0.12% chlorhexidine solution, 10% doxycycline solution, 3% sodium hypochlorite solution or 10% calcium chloride solution which was assessed using bacterial leakage test for a period of 60 days. The study results showed that the sealing ability of MTA was improved when combined with calcium chloride, sodium hypochlorite & doxycycline. This result was in accordance with those of **Hung et al** & **Bortoluzzi et al** wherein MTA-Calcium chloride & MTA-Sodium hypochlorite displayed lower microleakage values which could be attributed to the acceleration of the setting time of these mixtures. Also the increase in sealing ability of MTA-Doxycycline may be probably explained due to its antibacterial property of this material.

Nalini Desai et al (2016)[60] conducted an in vitro study to evaluate and compare the apical microleakage of three different root-end filling materials such as Mineral Trioxide Aggregate, Biodentine and Bioggregate. This study includes 30 extracted single rooted premolars which were decoronated, root canals were prepared and obturated with gutta percha and AH plus sealer. The 3mm of root end were resected, retrograde cavity was prepared and restored with the three tested materials which were then immersed in dye to assess the microleakage under stereomicroscope. The results of the study concluded that the Bioaggregate better sealing when compared to Biodentine and Mineral Trioxide Aggregate. The authors revealed that Bioaaggregate has nano-sized particles that achieve excellent adhesion to the dentinal walls of the root canal and the presence of gel-like calcium silicate hydrate as main structural component in calcium silicate based materials such as Bioaggregate and mineral Trioxide Aggregate that provides strength, hardness and sealing properties to the set material.

Wei Zhou et al(2017)[61] conducted a prospective randomised control study to compare the iRoot BP plus root repair material and MTA as root end filling materials in endodontic microsurgery. A total of 240 teeth were restored with MTA and iRoot BP plus as retrograde filling material after endodontic surgery were clinically and radiographically evaluated for a period of 12 months. The results of the study concluded that iRoot BP plus is comparable with MTA in clinical outcomes when used as root end filling material in endodontic microsurgery.

Shubha Chhaparwal et al (2017)[62] investigated the effect of chelating agents on sealing ability of Biodentine and Mineral Trioxide Aggregate when used as root-end filling materials. The methodology includes sixty human anterior teeth which were decoronated, instrumented, apically 3mm of the root-end was resected, retrograde cavities were prepared using ultrasonic tips. Teeth were then randomly divided into Group 1 & 2 (n=30) which were retrofilled with MTA & Biodentine respectively. Each group was further divided into three sub-groups A, B, C which are irrigated with 17% EDTA, 7%Maleic acid & 0.9% saline respectively. Then they were subjected to microleakage analysis at 24hrs, 7days & 14days using glucose infiltration technique. The results of the study showed that saline group demonstrated significant higher leakage than that of 17% EDTA & 7% Maleic acid in both MTA and Biodentine groups, 7% Maleic acid was able to remove the smear layer better than 17% EDTA and MTA had lower leakage values as compared to that of Biodentine when root-end cavities were irrigated with 7% Maleic acid. The authors attributed the minimal leakage with 7% Maleic acid irrigation was due to its efficient smear layer removal in the apical third. In the present study, MTA demonstrated better results due to superior marginal adaptation with root dentine by formation of tag-like structures.

MATERIALS AND METHODS

MATERIALS & METHODS

Source of samples:

Eighty extracted single rooted human mandibular premolars were collected from Department of Oral and Maxillofacial Surgery, Vivekanandha Dental College for Women, Tiruchengode.

Materials used:

1. Normal saline (Eurolife healthcare Pvt. Ltd, Roorkee, Uttarakhand, India)
2. 3% NaOCl irrigating solution (Vensons India, Bangalore, India)
3. 17% EDTA root conditioner (Glyde, Dentsply Maillefer, Ballaigues, Switzerland)
4. ProTaper F3gutta-percha points (Dentsply Maillefer, Ballaigues, Switzerland)
5. 17% EDTA solution (Smear clear, Sybron Endo)
6. AH plus root canal sealer (Dentsply de Trey GmbH, Konstanz, Germany).
7. GIC (GC corporation)
8. Mineral Trioxide Aggregate (MTA, Angelus)
9. Biodentine (Septodent, Saint-Maur-des-fossés, Cedex, France)
10. BioAggregate (iRoot BP plus, IBC, Vancouver, Canada)
11. 0.2% rhodamine B dye (Loba Chemie Pvt. Ltd., Jehangir Villa, Mumbai, India)

Armamentarium:

1. Diamond disk
2. Metal scale
3. Airotor Handpiece (NSK, Japan)
4. No.2 Round burs (Mani Inc., Japan)
5. Straight fissure carbide bur

6. Endobloc (Dentsply Maillefer, Ballaigues, Switzerland)
7. K- files – ISO 10 and 15 (Mani Inc., Japan)
8. Spreaders – ISO 25 and 20(Mani Inc., Japan)
9. Stailess steel scissor
10. Disposable 2.5ml syringe (DISPOVAN, Hindustan Syringes, Faridabad, India)
11. X-smart plus Endomotor and handpiece (Dentsply Maillefer, Ballaigues, Switzerland)
12. ProTaper Rotary Endodontic files (Dentsply Maillefer, Ballaigues, Switzerland)
13. Stainless steel ball burnisher
14. Stainless steel GP Condenser (Dispodent, Chennai, India)
15. Glass slab
16. Stainless steel cement spatula
17. Lentulospirals (Mani Inc., Japan)
18. Micromotor handpiece (NSK, Japan)
19. Spirit lamp
20. Stainless steel plastic filling instrument
21. Ultrasonic handpiece (NSK, Satelac)
22. Aceton S13 RD, S 14 LD Retrotips (NSK, Satelac)
23. Amalgamator
24. LED light curing unit (Woodpecker)
25. Humidity chamber
26. DSLR camera (NIKON, D3400)
27. Digimizer image analysing software
28. Autoclave
29. Stereomicroscope

S.NO	RETROGRADE FILLING MATERIAL	MANUFACTURER	COMPOSITION
1.	Glass Ionomer Light cured Universal restorative	GC Gold label, GC Corporation, Japan	Powder: Aluminium silicate glass
			Liquid: Polyacrylic acid, 3-hydroxy-ethyl methacrylate, 2,2,4- trimethy- hexamethy-dicarbonate, triethylene, glycol dimethacrylate
2.	Mineral Trioxide Aggregate	Angelus, Londrina, PR, Brazil	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, bismuth oxide
			Liquid: Water-based gel with thickening agents and water soluble polymers
3.	Biodentine	Septodent, Saint-Maur-des-fossés, Cedex, France	Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, iron oxide, zirconium oxide
			Liquid: Calcium chloride, hydrosoluble polymer
4.	BioAggregate	iRoot BP Plus, Innovative BioCeramix Inc., Vancouver, BC Canada.	Premixed Putty material: Tricalcium silicate, dicalcium silicate, zirconium oxide, tantalum pentoxide, calcium sulfate (anhydrous)

Method of collection of samples:

Eighty mandibular premolars with single canal were collected from the Department of Oral and Maxillofacial surgery, Vivekanandha Dental College for Women, Tiruchengode, which were indicated for extraction due to poor periodontal prognosis and orthodontic reasons.

Infection Control protocol for the teeth collected for this study:

Collection, storage, sterilization and handling of extracted teeth were followed according to the Occupational Safety and Health Administration (OSHA) and Centre for Disease Control and Prevention (CDC) recommendations and guidelines:

1. Handling of teeth was always done using gloves, mask and protective eyewear.
2. Teeth were cleaned of any visible blood and gross debris.
3. Distilled water was used in wide mouth plastic jars for initial collection.
4. Teeth were immersed in 10% formalin for 7 days, following which the liquid was discarded and the teeth were transferred into separate jars containing distilled water.
5. The initial collection jars, lids and the gloves employed were discarded into biohazard waste receptacles.
6. As and when the teeth were required, they were removed from the jars with cotton pliers and rinsed in tap water.

Inclusion Criteria:

- Teeth with completely formed roots.
- Teeth with normal anatomical roots.
- Absence of caries and root canal fillings.
- Patent single canal.
- Root canal with apical diameter of size 15 K file.

Exclusion criteria:

- Teeth with fractured roots.
- Multi-rooted teeth
- Teeth with open apices
- Calcified root canals
- Internal or external resorption
- Cracks on examination

PROCEDURE

Removal of external residual tissues:

Teeth were placed in 2.5% sodium hypochlorite solution for ten minutes to remove the soft tissues. Calculus was mechanically removed from the root surfaces using hand scalers. Teeth were again stored in fresh distilled water until use.

Preparation of the Root canals:

The crowns of all teeth samples were sectioned with a double faced diamond disc perpendicular to the long axis of the root at a standard measurement of 15mm from the apex **(FIG.1)**. Access was prepared on each tooth using high speed diamond burs with copious water spray. A size 10 K file was placed in the canal until it was visible at the apical foramen. The working length was determined by subtracting 1 mm from this measurement.

A size 15 K file was used to establish a reproducible glide path. Glyde root canal conditioner (Glyde, Dentsply Maillefer, Ballaigues, Switzerland) **(FIG.4)** was used as a lubricant for the files prior to insertion into the canals.

The canals were prepared using ProTaper Universal instruments upto size F3 instrument using X-Smart plus Endomotor and handpiece **(FIG.2 & 3)**. A size 10 K files was used to maintain apical patency between rotary file insertions. During instrumentation, all the canals were irrigated with 2.5 ml of 3% Sodium Hypochlorite solution between each instrument. The canals were finally rinsed with 3ml of 17% EDTA solution **(FIG.4)** and dried using F3 ProTaper paper points.

Obturation of the Root canals:

All the prepared root canals were obturated using lateral condensation technique. F3 gutta percha points along with AH plus sealer were used.

The AH plus sealer is available as two paste system **(FIG.5)**. According to the manufacturer's instruction, equal amounts both the pastes were dispensed onto the paper pad. The two pastes were mixed using a spatula until a uniform mix was obtained. The mixed sealer was coated onto the canal walls using lentulospiral. The lentulospiral was introduced into the root canal 2 to 3mm short of working length using low speed micromotor handpiece for 10 seconds and then slowly withdrawn from the canal.

F3 gutta percha points along with sealer were fitted into the prepared root canals of each tooth samples, ensuring adequate tug back and obturation was completed with lateral condensation method **(FIG.6)**. The excess gutta percha was sheared off. The access cavities were sealed coronally using Type 9 GIC restorative material.

The specimens then stored in an incubator at 37 degree centigrade in 100% humidity for 48 hours to allow the sealer to set completely.

Root end resection:

The apical 3mm of all the teeth were resected perpendicular to the long axis of the tooth using a straight fissure carbide bur on a high speed hand piece with copious water spray. **(FIG.7)**

Root end cavity preparation:

Root end cavities were prepared on all teeth to a depth of 3mm and width of 1mm using ultrasonic Satelac Retrotips (Aceton S13 RD, S 14 LD) in an Satelac NSK ultrasonic unit **(FIG.8 &9)**. The ultrasonic retrotip was used with light pressure in a brushing motion and the class I cavity was prepared parallel to the long axis of the tooth **(FIG.10)**. The root end cavity dimensions of all prepared teeth were checked with help of William's probe.

Filling of the root end cavities:

All the specimens were coated with two layers of clear nail varnish **(FIG.20)** except for the resected apical portion to seal all the other possible portals of communication with the root canals.

The eighty samples were then divided into four groups and retrograde filling was carried out as follows:

Group I: Glass Ionomer cement (GC corporation) (n=20) (FIG.11 & 12)

The light cured Universal restorative glass ionomer cement is available as powder and liquid system. The material is hand mixed on a paper pad according to the manufacturer's instructions and placed into the prepared root end cavity using a plastic instrument and tamped down with hand pluggers. Then they were cured for 20 seconds with Woodpecker's LED light curing unit.

Group II: Mineral Trioxide Aggregate (MTA Angelus) (n=20) (FIG.13 & 14)

The material is available as powder and liquid system. The material were dispensed in a clean glass slab and mixed using a spatula according to manufacturer's instructions. The mixed MTA were placed incrementally placed using an amalgam carrier. Then it compacted with hand pluggers and burnished with a ball burnisher to remove excess material and improve adaptation.

Group III: Biodentine (Septodent) (n=20) (FIG.15 & 16)

The material is available as Biodentine capsule and liquid pipettes. They were mixed according to the manufacturer's instructions and placed inside prepared root-end cavity.

Group IV: Bioaggregate (iRoot BP plus, IBC, Canada) (n=20) (FIG.17 & 18)

iRoot BP plus material is available as a syringe containing premixed bioaggregate putty material. The material was carried to the root end cavities using applicator tips fitted to the syringe containing the material.

Gauze moistened in distilled was used to wrap the specimens and they were stored in sealed containers **(FIG.19).** They were kept in incubator at 37 degree centigrade at 100% humidity for 24 hours.

Dye penetration and Stereomicroscopic analysis:

The specimens were immersed in 0.2% rhodamine B dye **(FIG.21 & 22)** and remained in the dye reservoir for 24 hours. Then they were removed from the dye reservoir and excess dye was rinsed off with running water for 15 minutes.

The specimens were sectioned longitudinally using diamond disc. The sliced specimens were then examined under a stereomicroscope at 30x magnification **(FIG.23)**. The extent of dye penetration was measured in millimeter using Digimizer Image Analysis software **(FIG.24)**.

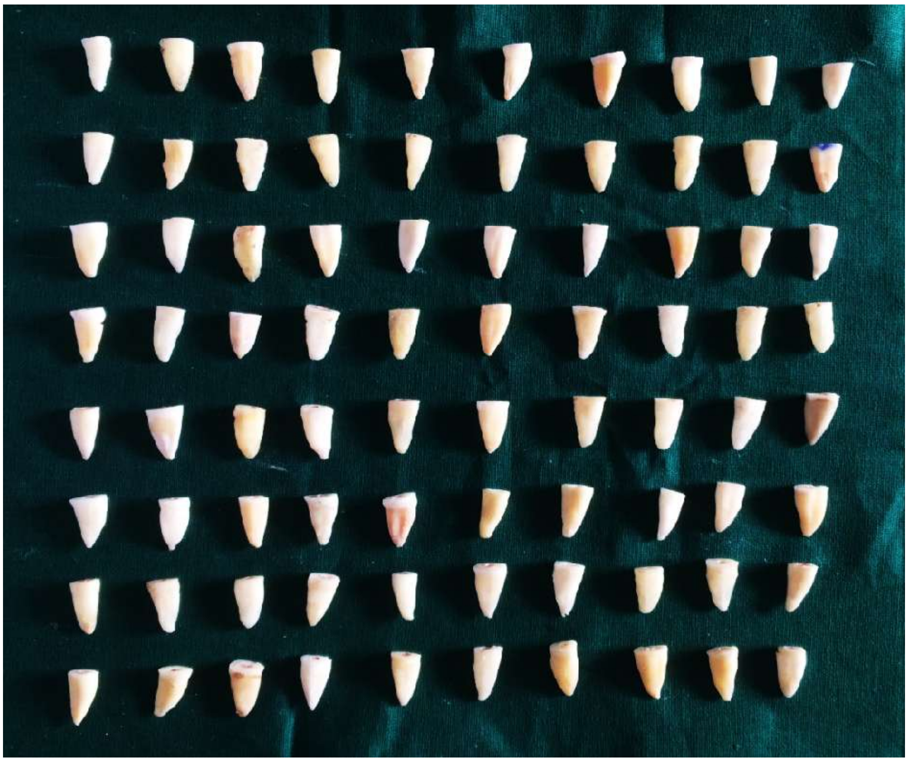


FIG.1: DECORONATED HUMAN SINGLE ROOTED MANDIBULAR PREMOLAR TEETH

FIG.2: X-SMART PLUS ENDOMOTOR AND HANDPIECE

FIG.3:PREPARATION OF THE ROOT CANALS OF THE SAMPLES

FIG.4: IRRIGATION REGIMEN USED IN ROOT CANAL PREPARATION

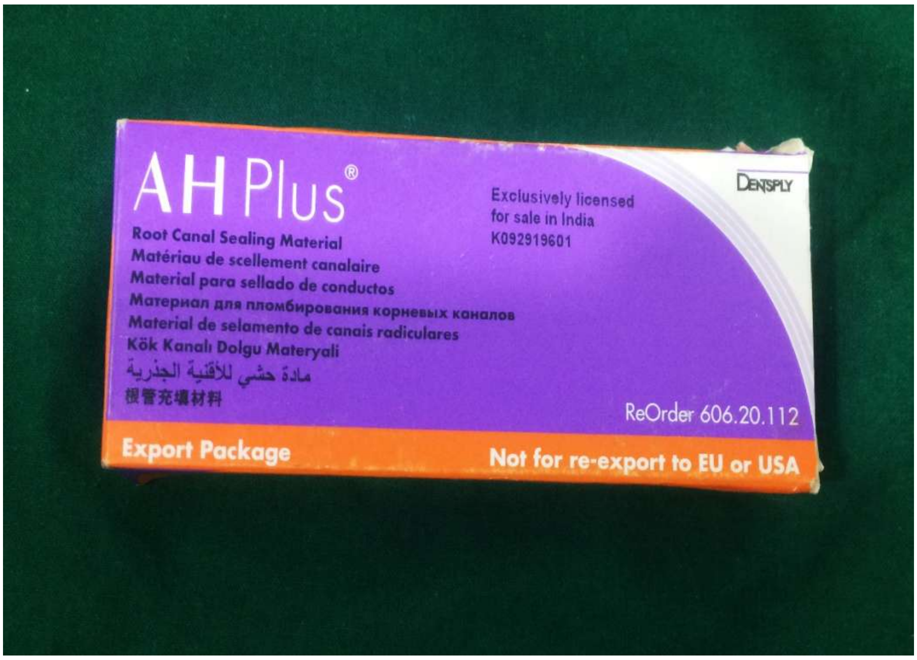


FIG.5: AH PLUS ROOT CANAL SEALER

FIG.6: OBTURATION OF THE ROOT CANALS OF THE SAMPLES

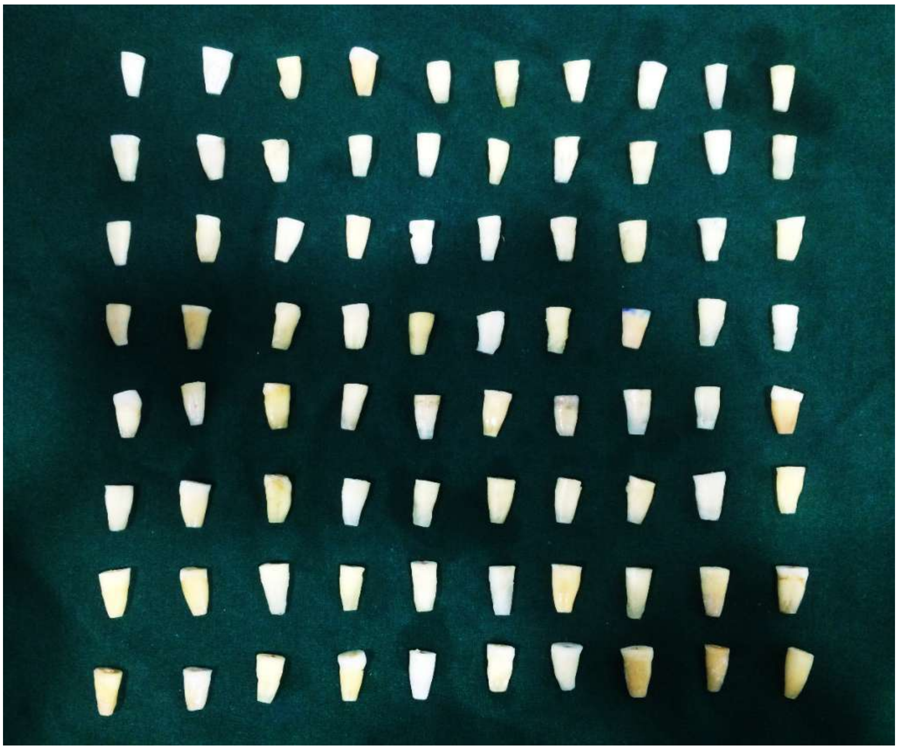


FIG.7: ROOT-END RESECTED SAMPLES

FIG.8: ULTRASONIC SATELAC RETROTIPS (ACETON S13 RD, S 14 LD)

FIG.9: SATELAC NSK ULTRASONIC UNIT

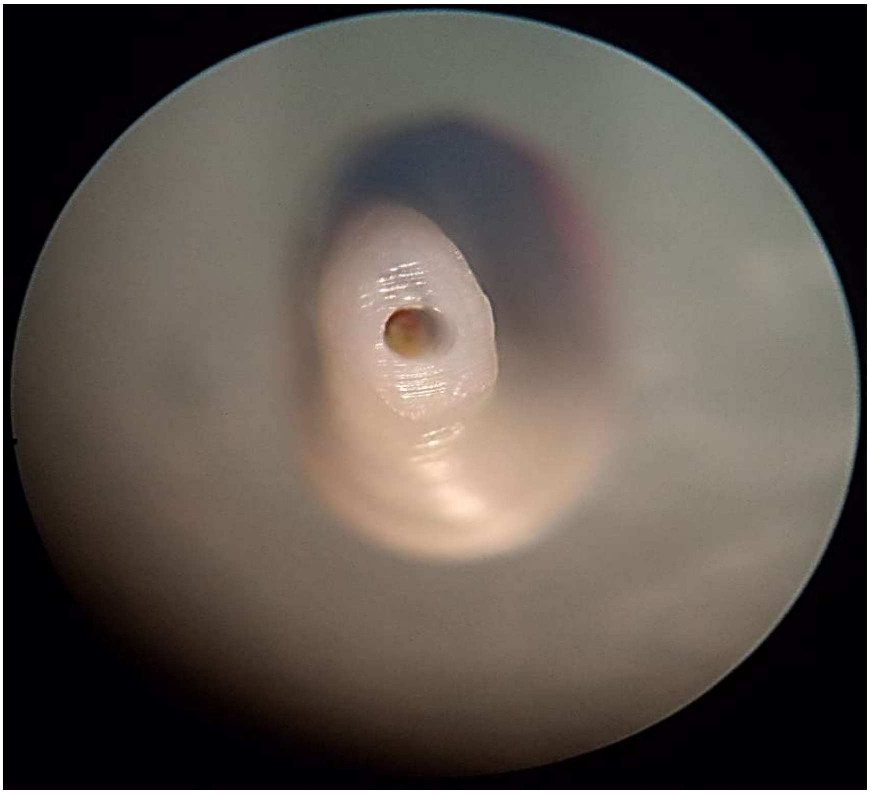


FIG.10: RETROGRADE CAVITY OF THE SAMPLES

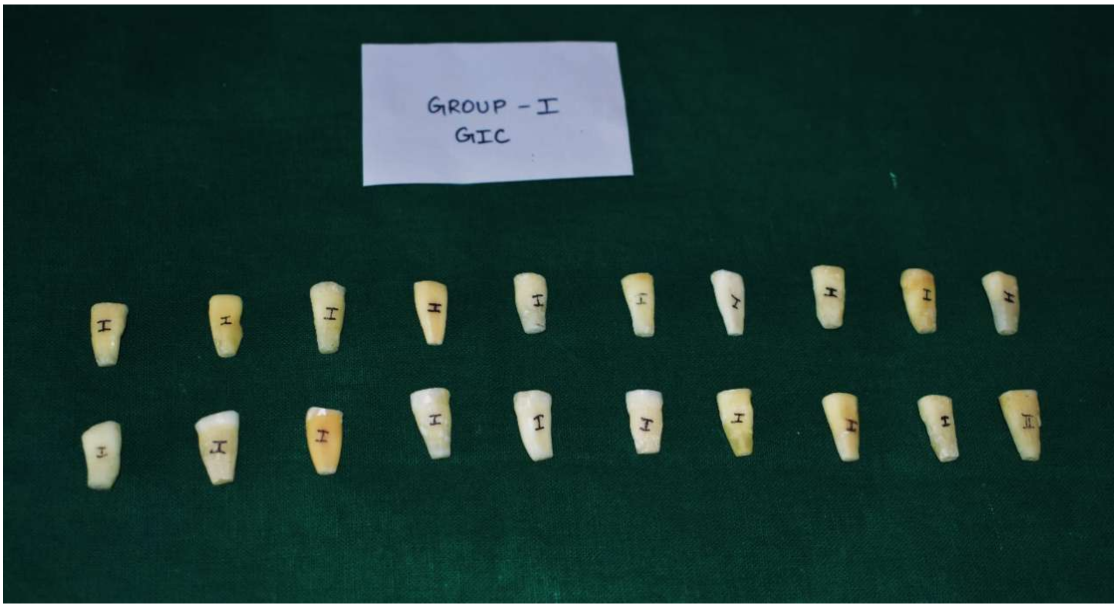


FIG.11: GROUP I SAMPLES RETRO-FILLED WITH LIGHT CURED GLASS IONOMER CEMENT

FIG.12: LIGHT CURED GLASS IONOMER CEMENT (GC CORPORATION)

FIG.13: GROUP II SAMPLES RETRO-FILLED WITH MINERAL TRIOXIDE AGGREGATE

FIG.14: MINERAL TRIOXIDE AGGREGATE (MTA, ANGELUS)

FIG.15: GROUP III SAMPLES RETRO-FILLED WITH BIODENTINE

FIG.16: BIODENTINE (SEPTODENT)

FIG.17: GROUP IV SAMPLES RETRO-FILLED WITH BIOAGGREGATE

FIG.18: BIOAGGREGATE PUTTY (IROOT BP PLUS, IBC, CANADA)

FIG.19: RETROFILLED SAMPLES

FIG.20: NAIL VARNISH

FIG.21: RHODAMINE B DYE IN POWDER FORM

FIG.22: 0.2% RHODAMINE B DYE SOLUTION

FIG.23: STEREOMICROSCOPE

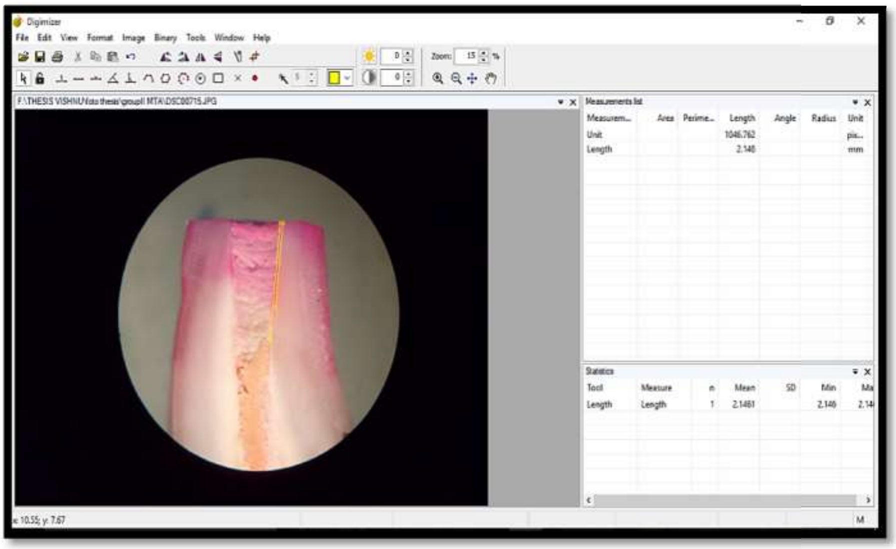


FIG.24: DYE PENETRATION OF SAMPLES EVALUATED IN MILLIMETERS USING IMAGE ANALYSIS SOFTWARE

RESULTS

STEREOMICROSCOPIC IMAGES OF DYE PENETRATION IN SPECIMENS REPRESENTING FROM FOUR TESTED GROUPS

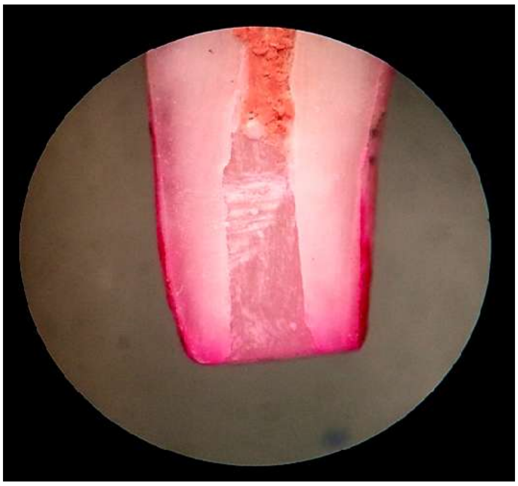


FIG.25: GROUP I (GLASS IONOMER CEMENT) sample at 30x magnification

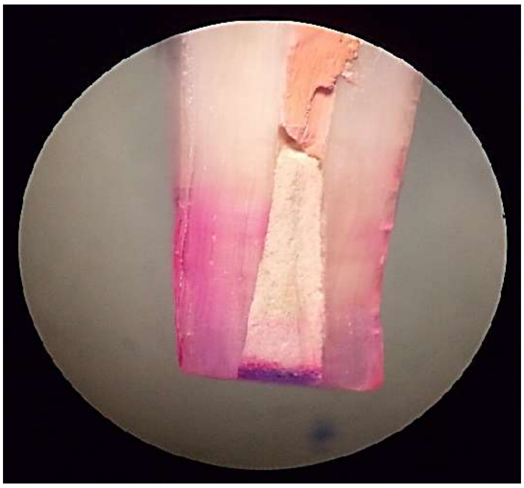


FIG.26: GROUP II (MINERAL TROXIDE AGGREGATE) sample at 30x magnification

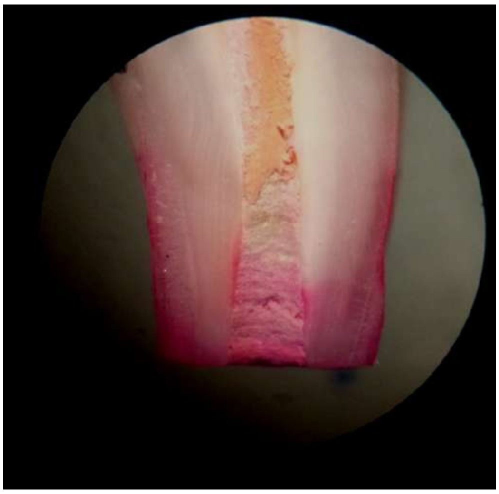


FIG.27: GROUP III (BIODENTINE) sample at 30x magnification

FIG.28: GROUP IV (BIOAGGREGATE) sample at 30x magnification

TABLE 1:

THE DEPTH OF DYE PENETRATED IN THE SAMPLES OF EACH GROUP IN MILLIMETERS FROM APICAL END TOWARDS THE CERVICAL END

SAMPLE NO.	GROUP I (GIC)	GROUP II (MTA)	GROUP III (BIODENTINE)	GROUP IV (BIOAGGREGATE)
1.	1.9mm	0.3mm	2.4mm	0.8mm
2.	2.3mm	0.2mm	2.0mm	0.4mm
3.	2.5mm	1.6mm	2.0mm	0.4mm
4.	2.4mm	0.5mm	0.9mm	0.2mm
5.	1.4mm	1.0mm	2.5mm	0.1mm
6.	2.7mm	0.2mm	2.1mm	0.1mm
7.	2.5mm	0.7mm	3.0mm	0.1mm
8.	3.0mm	0.3mm	1.9mm	0.3mm
9.	2.7mm	0.4mm	3.0mm	0.4mm
10.	2.0mm	2.1mm	3.0mm	0.2mm
11.	1.9mm	1.2mm	0.6mm	0.1mm
12.	3.0mm	0.4mm	0.2mm	0.3mm
13.	2.6mm	0.6mm	1.3mm	0.1mm
14.	2.0mm	1.0mm	2.7mm	0.4mm
15.	2.7mm	0.8mm	1.6mm	0.5mm
16.	1.8mm	1.3mm	0.9mm	0.4mm
17.	2.5mm	0.7mm	2.3mm	0.1mm
18.	2.6mm	0.5mm	1.5mm	0.2mm
19.	2.8mm	0.6mm	1.2mm	0.3mm
20.	3.0mm	0.9mm	2.8mm	0.4mm

TABLE 2:

APICAL SEALING ABILITY OF FOUR RETROGRADE FILLING MATERIAL ANALYSED BY ANOVA TEST AT A SIGNIFICANCE LEVEL OF 0.05

		Apical Sealing Ability				
Group	**N**	**Mean**	**SD**	**Std. Error**	**ANOVA**	**p**
GIC	20	2.42	0.45	0.10	64.83	< 0.001**
MTA	20	0.77	0.49	0.11		
BIODENTINE	20	1.90	0.85	0.19		
BIOAGGREGATE	20	0.29	0.18	0.04		
Total	80	1.34	1.01	0.11		

The mean values and the standard deviations of dye penetration in each group are shown in Table 2. ANOVA test concludes that the values were highly statistically significant among all four tested materials ($p<0.05$), where Group IV shows least dye penetration (0.29mm) and Group I was found to have highest dye penetration (2.42mm).

TABLE 3 & 4:

APICAL SEALING ABILITY OF FOUR RETROGRADE FILLING MATERIALS ANALYSED BY TUKEY B POST HOC TEST

Multiple Comparisons - Dependent Variable: Apical Sealing Ability - Bonferroni

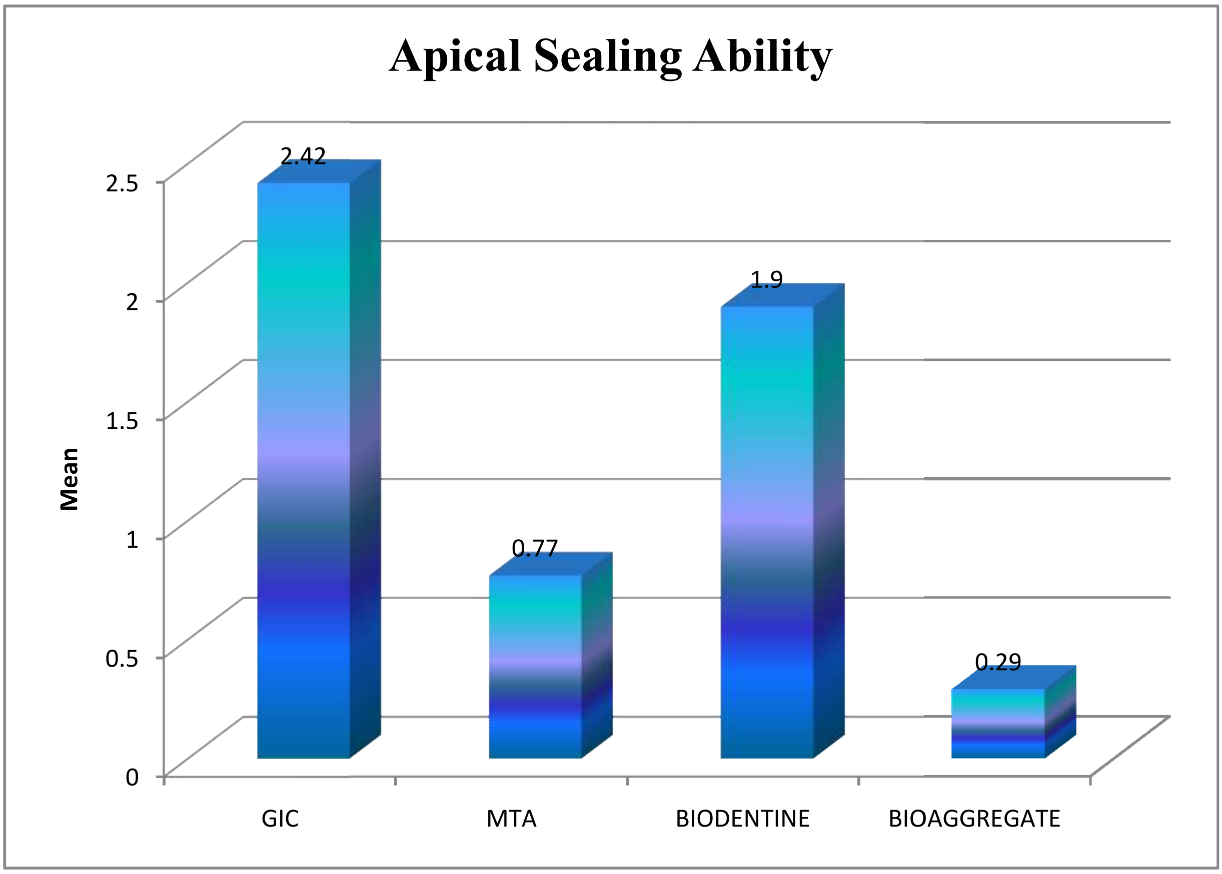
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GIC	MTA	1.6500(*)	.17259	.000	1.1824	2.1176
	BIODENTINE	.5200(*)	.17259	.021	.0524	.9876
	BIOAGGREGATE	2.1250(*)	.17259	.000	1.6574	2.5926
MTA	GIC	-1.6500(*)	.17259	.000	-2.1176	-1.1824
	BIODENTINE	-1.1300(*)	.17259	.000	-1.5976	-.6624
	BIOAGGREGATE	.4750(*)	.17259	.044	.0074	.9426
BIODENTINE	GIC	-.5200(*)	.17259	.021	-.9876	-.0524
	MTA	1.1300(*)	.17259	.000	.6624	1.5976
	BIOAGGREGATE	1.6050(*)	.17259	.000	1.1374	2.0726
BIOAGGREGATE	GIC	-2.1250(*)	.17259	.000	-2.5926	-1.6574
	MTA	-.4750(*)	.17259	.044	-.9426	-.0074
	BIODENTINE	-1.6050(*)	.17259	.000	-2.0726	-1.1374

* The mean difference is significant at the .05 level.

Group	N	Subset for alpha = .05			
		1	2	3	4
BIOAGGREGATE	20	.2900			
MTA	20		.7650		
BIODENTINE	20			1.8950	
GIC	20				2.4150

The intergroup comparisons was evaluated using Tukey B Post hoc test was shown in Tables 3 & 4. The results obtained by this study revealed that the mean microleakage was significantly higher in Group I Glass Ionomer Cement, followed by Group III Biodentine, Group II Mineral Trioxide Aggregate and with least microleakage was seen in Group IV BioAggregate.

BAR DIAGRAM SHOWING MEANMICROLEAKAGE VALUES (in mm) OF FOUR RETROGRADE FILLING MATERIALS



DISCUSSION

DISCUSSION

The objective of periapical surgery is to surgically maintain a tooth that has an endodontic lesion which cannot be resolved by conventional endodontic treatment. This goal is achieved by root-end resection, root-end cavity preparation and a bacteria-tight closure of the root canal system at the cut root-end with a retrograde filling.[63]

Most endodontic failures occurs as a result of leakage of irritants & microbes from the infected root canals. The success of periradicular surgery is directly dependent on the good apical seal, using a well adapted root-end filling material. These material are intended to prevent the leakage of potential irritants from the root canal system into the periradicular tissues.[64] So, an ideal retrograde filling material must have good adhesion to the canal wall providing an adequate apical seal. It should also be biocompatible and able to possess osteoinductive or osteoconductive qualities which will accelerate the healing process at the periapical area and reduce the incidence of failures. Hence in this study we assessed the apical sealing ability of four different retrograde filling materials to the root dentine.

In the present study, single rooted mandibular premolars were used with crowns removed at the cementoenamel junction for standardization of specimens as it eliminated some variables, such as the anatomy of the coronal area and the access to the root canal. Rotary system (ProTaper) was used for root canal preparation in all groups, as it allows a more uniform preparation without obvious procedural errors and the canals prepared up to the size F3 (MAF) which is equal to ISO 030 tip size. Sodium hypochlorite was used as a canal irrigant because of its lubricant, antimicrobial, organic tissue dissolving properties. The final irrigation was done with 17% EDTA solution and the samples were obturated with F3 gutta percha with AH Plus sealer.

AH Plus is epoxy resin based endodontic sealers which can be used with gutta-percha to obtain a three dimensional filling. Due to its flowability, the epoxy resin-based sealers will penetrate deeper into the dentinal tubules and its long polymerization time enhances the mechanical interlocking of the sealer to root dentine. These properties further lead to greater intertwining of the sealer with dentin structure and together with the cohesion among the cement molecules, it provides greater adhesiveness and resistance to dislodgment from root dentin.[65] So, in our current study AH Plus root canal sealer was used to carry out obturation of the root canal space.

The term microleakage is defined as the passage of bacteria, fluids and chemical substances between the restorative materials and the tooth. It is an estimate of the quality of seal obtained by the filling materials and it can be measured by allowing a tracer to penetrate through the filled cavity. Commonly used tracers include dyes, radioisotopes, bacteria and bacterial by-products. Several methodologies can be employed to assess the apical microleakage which often includes dye penetration, fluid filtration, bacterial leakage and protein leakage.[66] There is no evidence of superiority of any certain method. **Chong et al in 1995** compared the penetration of tracers and other assessment methods for the efficacy of sealing ability of root-end filling materials. The findings of their study concluded that bacterial penetration and dye penetration methods yielded better results. The dye immersion technique was introduced by **Grossman in 1939** which is a passive method that depends on the phenomenon of capillarity, whereby the dye penetrates any space between the root-end filling & the dentinal wall of the root canal. Dye penetration method is most popularly used for microleakage studies as the dyes are cheap, safe, readily available, relatively easy to be stored & used and most importantly their penetration can be evaluated quantitatively.[67]

Dye penetration should be considered as an indicator of the potential for leakage. This is because according to **Torabinejad et al (1994)**[31] a filling material able to resist the penetration of small molecules such as dyes, would have the potential to resist the penetration of larger bacteria and their by-products. So, Dye penetration method was employed in our study as it yields reliable results.

Removal of 3-4mm of root-end is common during periradicular surgery and is usually required to eliminate anatomical irregularities and contaminated (biofilms, bacteria & endotoxins) radicular hard tissues. Root-end resection was carried out with a high-speed rotating bur & coolant, minimizing heat generation and prevents the development of root fractures.[68]

Root-end resection can be done at different planes ie. 30^0, 45^0 and 90^0 to the long axis of the tooth. Among these the most accepted is 90^0, as it least affects the adaptability of root-end material and minimizes the leakage that might occur through the cut dentinal tubules whereas 30^0 & 45^0 resection angles have a disadvantages of leading to open dentinal tubules, more mechanical stresses, loss of dentine-cementum bone which results in compromised healing after periapical surgery.

Numerous anatomical variations such as apical ramifications and lateral canals occurs mostly in the apical 3mm of the root-end. Resection of 3mm of the root-end reduces 98% of apical ramifications and 93% of lateral canals.[69] So, in our current study, 3mm of root tip resection perpendicular (90^0) to the long axis of the tooth was performed to eliminate apical ramifications and lateral canals thus reducing the number of open dentinal tubules and leakage at the resected root-end.

Retrograde cavities are prepared at the resected root-end with rotary burs in microhand piece or using ultrasonic instruments. The goal of root-end cavity preparation is to remove the intracanal filling material & irritants and to create a cavity that can be properly filled. The ideal root-end preparation can be defined as a class I cavity at least 3mm into root dentine, with walls parallel to and coincide with the anatomic outline of the root canal space.[70]

To prepare root-end cavities during surgical endodontic procedures, ultrasonic instruments were used especially in teeth where uniting anastomoses or isthmi are present. The use of ultrasonics in endodontics was first introduced by **Richman in 1957**. He used modified ultrasonic periodontal chisel scaler for root canal debridement and apicoectomy. In 1944, **Carr** introduced retrotips specifically designed for root-end cavity preparation which can be used during periapical surgery.

The ultrasonic retrotips are made up of stainless steel or stainless steel with diamond coating or zirconium coating. In a study done by **H.Ishikawa et al in 2003**, they evaluated the root-end cavity prepared using ultrasonic retrotips, the authors concluded that use of ultrasonic retrotips with diamond or zirconium coating takes less time for preparing retrograde cavities and the retro cavities prepared were more accurate & these tips have more efficient cutting abilities than compared to rotary burs.[71]

A variety of tips were marketed to accommodate virtually all access situations and designed to penetrate from 3 to 9 mm. During usage, retrotips are placed in long axis of the root so that the walls of the preparation will be parallel and encompass about 3mm of apical morphology. As the piezoelectric crystal in the handpiece is activated, the energy is transferred to the ultrasonic tip, which then moves forward & backward in a single plane.[72]

The advantages of ultrasonic tips over burs are:

- Smaller apical preparations
- Cleaner apical preparations
- Easier isthmus preparation between the exits of apical canals
- Easier access to root tips
- Lesser strain and fatigue for the operator.

The most relevant clinical advantage is the enhanced access to root-ends in a limited working space. They also provide better centered root-end preparation that follows the original path of the root canal which lessens the risk of lateral perforation. Furthermore, the ultrasonic retrotips produced less smear layer in a retro-end cavity compared to a slow speed handpiece. Moreover, the refinement of cavity margins that were obtained with the ultrasonic tips will positively affect the delivery of materials into the cavities and enhance their seal.[73]

The retrograde cavity preparation should be ideally 3mm as more than that does not have any greater benefits whereas lesser depth has negative effect on the long term success of apical seal. The preparation depth of 3mm decreases the leakage which is attributed to the occlusion of apical dentinal tubules by retrograde filling materials. Hence in the present study, optimum depth of 3mm retrograde cavities are prepared using diamond coated ultrasonic retrotips - Aceton S13 RD, S 14 LD Retrotips (NSK, Satelac).

A variety of dyes are used for dye leakage studies which includes India Ink, erythrosine B solution, aqueous solution of fuchsin, fluorescent solution, silver nitrate, Methylene blue, rhodamine B and others.

Dye leakage studies are dependent on numerous variables such as the immersion period of the specimens into the marker, the time of immersion; the use or non-use of negative pressure (vaccum) to remove air trapped within filling gaps; total or partial immersion of the specimens into the dye; type of seal; number of specimens; volume of the marker; position of specimens during immersion; & especially the type of material used.[74]

In a study done by **Starkey, Anderson & Paskley** on effects of dye pH on apical leakage, they emphasized the use of dye solutions with neutral pH. So, it seems reasonable to use rhodamine B dye of neutral pH in the present study so as to avoid variables inherent to the methods.

The depth of penetration of dyes into the tooth structure varies according to the amount of air trapped within the canal. In the present study, vaccum was not used and dye penetration occurred passively; so as to resemble the clinical scenario. Methylene blue is widely used in many dye leakage studies. But it has many disadvantages such as methylene blue when comes in contact with alkaline filling material produces discolouration. This occurs as a result of hydrolysis of methylene blue, leading to formation of a clear compound named thioxine.[75]

MTA presents with high pH (12.5) and contains calcium oxide which leads to calcium hydroxide formation when it contacts with water, revealing discoloration by methylene blue. So, in order to assess the sealing ability of MTA, the dye solutions that do not negatively affect the alkalinity of their marking capability is used.

In our study, rhodamine B dye which has more advantages than methylene blue was used. Rhodamine B, an organic dye which is classified as xanthenic dye is a watersoluble fluorescent dye which is easily detectable even in low concentrations, moves freely along the interface, low toxicity, stable in an aqueous environment, stable in varying pH and non-destructive to the substrate or material in contact. It has greater diffusion on human dentin than methylene blue. According to **Franci et al**, the molecules of rhodamine B dye are nanometric and are optimal to stimulate enzymes and toxins of leakage resulting from bacterial metabolism. Another important feature that makes it an alternative to methylene blue is their neutral pH and they are not affected by alkaline filling material. **(Tanomaru Filho 2005 et al & Moraes et al 2005)[76]**

The recommended times of exposure to dyes in leakage studies have ranged from 2 hours to 30 days. In our investigation the teeth were left in dye solution for 24 hours.

The dye penetration can be measured spectrophotometrically or linearly. Linear measurements can be studied after longitudinal splitting; cross sectioning or by clearing of the specimens. But in a study comparing longitudinal splitting, cross sectioning and cleaning of the specimens, greater dye penetration was recorded with the samples that underwent longitudinal splitting.**(M.K.Wu & Wesselink, 1993)[74]** In our study, linear measurement of penetrated dye is evaluated after longitudinal splitting of the samples with diamond disc and viewed under stereomicroscope at 30 x magnification. The amount of dye penetration in all four groups were evaluated in millimetres using Digimizer Image Analysis Software.

The results of the study showed that all materials tested exhibited microleakage. The mean dye penetration of Group IV-BioAggregate (0.29mm) showed lesser values **(FIG.28)** indicating better sealing with the root dentine than compared with that of the groups MTA (0.77mm) **(FIG.26)**, Biodentine (1.90mm) **(FIG.27)**, and GIC (2.42mm) **(FIG.25)**. There was significant difference in the dye leakage values among the four tested root-end filling materials. However the microleakage values of Group II-MTA are comparable with that of Group IV-BioAggregate. The highest significant linear leakage was shown by Group I-GIC followed by Group II-Biodentine.

The higher microleakage values produced by Group I-GIC and Group II-Biodentine may be attributed to high solubility possessed by these two materials. Lack of solubility is one of the important factors that a root-end filling material should possess to reduce the microleakage and ultimately preventing migration of bacteria and endotoxins into the periradicular tissues.

Glass ionomer cement is a material with universal properties and it is used as dentin substitute. The good marginal seal provided by this cement is due to its ability to bond chemically with the tooth structure. It is also shown to possess antibacterial activity due to slow release of fluoride. In confocal microscopic study done by **Chong et al in 1991**[77] showed that the sealing ability of light cured GIC possessed better marginal adaptation as a retrograde filling material. Hence this material was used in this study. However in this study, compromised sealing was produced by this material that may be due to dissolution of the material in tissue fluids and it being technique sensitive. This was in congruence with the study done by **King in 1990** on longitudinal evaluation of the seal of endodontic retrofillings.

Biodentine is a calcium silicate based cement which is similar to MTA in its composition and water chemistry. In addition it has increased physiochemical properties like short setting time, high mechanical strength and new pre-dosed capsule formulation makes it easy to handle and can be considered superior to MTA. In a study done by **Kokate et al in 2012**[78] on comparing microlakage of MTA, GIC & Biodentine using dye penetration method, Biodentine showed significantly lesser leakage than the other two materials. Also in the studies done by **Prasanti Kumari Pradhan et al (2015)**[49] and **Ankita Khandelwal et al (2015)**[53] on assessing the sealing ability of retrograde filling materials, Biodentine showed better sealing with significantly less microleakage than GIC and MTA. The better sealing of Biodentine is due to its ability in forming of tag-like structures when it comes in contact with dentine and its smaller particle size makes it well adapt to the cavity surface. Also the set Biodentine has less porosity and pore volume when compared to MTA.

In our study, the second high dye penetration values were seen with Group III- Biodentine specimens. The cause for Biodentine to show high microleakge may be due to its higher solubility rate. In a study done by **Sawsan et al (2015)**[79] on analysing the solubility, pH changes and leaching elements in white MTA & Biodentine, the specimens with Biodentine exhibited higher solubility, prolonged alkalinity and increased calcium release than white MTA. This could also be attributed to its low water sorption affecting its solubility. So due this probable reason, in the current study Biodentine specimens exhibited more microleakage than MTA.

MTA has been investigated and used as a root end filling material since its introduction. It has favourable properties suitable for root-end filling material such as excellent sealing ability, biocompatibility, good compressive strength (67MPa), insoluble in fluids, radiopacity and antibacterial effect. The results of the current study showed GroupII-MTA has less dye leakage than GroupI- GIC & GroupIII- Biodentine and its marginal seal produced can be comparable to that of GroupIV- BioAggregate.

The reason for this may be due to formation of hydroxyapatite like crystals at the interface between material and canal wall due to which the material shows better adhesion preventing the penetration of the dye and less microleakage.

The white-MTA (Angelus) used in the study has significant sealing properties comparable with that of gray-MTA. The smaller particle size of white-MTA results in greater specific surface area leading to increase in the wetting volume, water binding capacity and hydration rate. Moreover, MTA undergoes setting expansion when cured in moist environment due to its hydrophilic nature and thus the presence of moisture does not affect its setting or the properties during periapical surgery.[80]

The specimens of GroupIV- BioAggregate exhibited least mean dye leakage values (0.29mm) among all four tested materials. The hermetic seal obtained by this new bioceramic based BioAggregate material was due to its nano-sized particles that achieve excellent adhesion to the dentinal walls of the root canal, its hydrophilic nature and does not shrink during setting process. It exhibits excellent physical properties.

According to this study, GroupIV- BioAggregate produced better sealing ability as retrograde filling material and nearly closer results were obtained by GroupII- Mineral Trioxide Aggregate. Even several in vitro studies have compared MTA & iRoot BP Plus BioAggregate material and found that they exhibited similar characteristics. This is because they both have nearly same composition. In both Mineral Trioxide Aggregate and BioAggregate, the main structural component is gel-like calcium silicate hydrate which provides strength, hardness and good sealing properties to these set materials.[44, 81]

SUMMARY

SUMMARY

The objective of this study was to compare and evaluate the apical sealing ability of four retrograde filling materials namely GIC (GC Corporation), Mineral Trioxide Aggregate (MTA, Angelus), Biodentine (Septodent, Saint-Maur-des-fossés, Cedex, France) and BioAggregate (iRoot BP plus, IBC, Vancouver, Canada) by dye penetration method using stereomicroscope.

Eighty extracted human single rooted premolars with single canal were collected and decoronated at cementoenamel junction (CEJ). Cleaning and shaping was done with ProTaper rotary file system (Dentsply Maillefer, Ballaigues, Switzerland) and 17% EDTA root conditioner (Glyde, Dentsply Maillefer, Ballaigues, Switzerland). Irrigation regimen used in this study were Normal saline (Eurolife healthcare Pvt. Ltd, Roorkee, Uttarakhand, India), 3% NaOCl irrigating solution (Vensons India, Bangalore, India) and 17% EDTA solution (Smear clear, Sybron Endo). The obturation of the root canals of the specimens were carried out using ProTaper F3 gutta-percha points (Dentsply Maillefer, Ballaigues, Switzerland) and AH plus root canal sealer (Dentsply de Trey GmbH, Konstanz, Germany).Thereafter the apical 3mm of the root-end of the specimens were resected using diamond disc and retrograde cavities were prepared to a depth of 3mm using ultrasonic Satelac Retrotips (Aceton S13 RD, S14 LD) in an Satelac NSK ultrasonic unit.

Teeth were then randomly divided into four groups of 20 samples each according to the material used for retrograde fillings. In Group I, the samples were retrofilled with light cured Glass Ionomer Cement (GC Corporation), Group II with Mineral Trioxide Aggregate (MTA, Angelus), Group III with Biodentine (Septodent, Saint-Maur-des-fossés, Cedex, France) and Group IV with BioAggregate (iRoot BP

plus, IBC, Vancouver, Canada). These retrofilled samples of four groups were stored in sealed containers at 37 degree centigrade & 100% humidity for 24 hours.

The specimens were then immersed in 0.2% rhodamine B dye for 24 hours, washed, dried and sectioned longitudinally. These sectioned specimens were analysed under a stereomicroscope and dye penetration was evaluated in millimetres for each group using Digimizer Image Analysis Software.

The results of this study revealed that Group IV samples retrofilled with BioAggregate showed least dye penetration among all four groups. The second least dye penetration was observed in Group II samples retrofilled with Mineral Trioxide Aggregate. The highest dye penetration was seen with Group I samples with GIC followed by Group III Biodentine. This indicates that better apical sealing was produced by new bioceramic material BioAggregate.

CONCLUSION

CONCLUSION

Under experimental conditions and within the limitations of the study,

- All the four tested materials showed dye penetration indicating microleakage.
- The least dye penetration values were seen in GroupIV- BioAggregate specimens followed by GroupII- MTA, GroupIII- Biodentine and highest values were with GroupI- Glass Ionomer Cement.
- The new bioceramic based material, BioAggregate showed better apical sealing ability as a root-end filling.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Chaurasiya S, Yadav G, Tripathi AM, Dhinsa K. Endodontic Failures and its Management: A Review. Int J Oral Health Med Res, 2016, Vol: 2, Issue: 5, P: 144-148.
2. Tabassum S, Khan FR. Failure of endodontic treatment: The usual suspects. Eur J Dent, 2016, Vol: 10, P: 144-7.
3. Priyanka.S.R, Dr.Veronica, A Literature Review of Root-End Filling Materials. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), Volume 9, Issue 4, Sep.- Oct. 2013, P: 20-25.
4. HC Seedat, PJ van der Vyver. An in-vitro comparison of microleakage between three calcium silicate cements and amalgam. SADJ, April 2016, Vol: 71, No 3, p100 - p105.
5. Claudio Poggio, Marco Lombardini, Conti Alessandro, Rindi Simonetta. Solubility of Root-end filling materials: A comparative study, Journal of Endodontology. Vol: 33, Issue: 9, 2007, P: 1094-1097.
6. Wu MK, Dean SD, Kersten HW. A quantitative microleakage study on a new retrograde filling technique. Int Endod J, 1990, Vol: 23, P: 245-9.
7. Bhargava S, Chandra S, Chandra S: A comparison of tissue reactions to potential retrograde root filling materials. Endodontology, 1999, Vol:11, P: 8-13.
8. Al-Nazhan S, Sapounas G, and Spangberg LSW: In vitro study of the toxicity of a composite resin, silver amalgam and cavit. J Endod, 1988, Vol: 14, P:236-8.
9. John.M.Yaccino, William.A.Walker, David.L.Carnes, William.G.Schindler , Longitudinal microleakage evaluation of Super-EBA as a root-end sealing material, Journal of Endodontics, 25(8), 1999, 552-554.

10. B.S.Chong, T.R.Pitt Ford, S.P.Kariyawasam, Short term response to potential root-end filling materials in infected root canals. International Endodontic journal,(30), 1997, 240-249.
11. Divya. D, Vedavati. B, Roopa. R. Nadig, Preeti. M. Tippashetty. All is well that ends well; A review on root-end filling materials. Journal of Dental Sciences and Research, Vol: 5, Issue: 2, September 2014.
12. Gupta SK, Saxena P, Pant VA, Pant AB. Adhesion and biologic behavior of human periodontal fibroblast cells to resin ionomer Geristore: a comparative analysis. Dent Traumatol 2012; Nov 6 [Epub ahead of print]. DOI:10.1111/edt.12016.
13. Kamran Safavi, Reza Kazemi, Dudley Watkins, Adherence of enamel matrix derivatives on the root-end filling materials, Journal of endodontics, Vol:25, Issue:11, 1999, P: 710-712.
14. Dong Sung-park, suh-Jin Sohn, Tae-Seok oh, Hyun-Mi Yoo, Chan-Je Park, Soon-Ho Yim, Young-Kyoo Lee, Seung-Bum Kye, An electrochemical study of the sealing ability of three retrofilling materials, Journal of Korean Academy for Conservative Dentistry, Vol: 29, Issue: 4, 2004, P: 365-369.
15. Witherspoon DE, Gutmann JL, Analysis of the healing response to gutta-percha and Diaket when used as root-end filling materials in periradicular surgery, International Endodontic Journal, Vol: 33, 2000, P: 37-45.
16. Kelvin. C.Y., Tay, Bethany. A., Loushine, Cindy Oxford, Rishi Kapur, Caroline. M. Primus, James. L. Gutmann, Robert. J. Loushine., David. H. Pashley, Franklin. R. Tay. In Vitro evaluation of Ceramicrete-based Root-end filling material. Journal of Endodontology, Vol: 33, Issue: 12, 2007, P: 1438-1443.
17. Giovana Ribeiro De Martins, Claudio Antonio Talge Carvalho, Marcia Carneiro Valera, Luciane Dias De Oliveira, Leonardo Buso, Alessandra Sverberi Carvalho.

Sealing Ability of Castor Oil Polymer as a Root-end Filling Material. J Appl Oral Sci. 2009, Vol: 17, Issue: 3, P: 220-3.

18. Mahmoud Torabinejad, C. U. Hong, F. McDonald and T. R. Pitt Ford. Physical and Chemical Properties of a New Root-End Filling Material. Journal of Endodontics, Vol. 21, No. 7, July 1995.
19. B.S. Chong, T.R. Pitt Ford, M.B. Hudson. A prospective clinical study of Mineral Trioxide Aggregate and IRM when used as root-end filling materials in endodontic surgery. International Endodontic Journal, Vol: 36, P: 520-526, 2003.
20. Masoud Parirokh and Mahmoud Torabinejad. Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part I: Chemical, Physical, and Antibacterial Properties. JOE, Volume 36, Number 1, January 2010.
21. A.R.Atmeh, E.Z.Chong, G.Richard, F.Festy, T.F.Watson, Dentin-cement interfacial interaction: Calcium silicates and Polyalkenoates, Journal of dental research, Vol: 91, Issue: 5, 2012, P: 454-459.
22. Trond Bjorvik Osen, Ina Iselin Astrup, Carl Haavard Knutsson. Biodentine as root-end filling, 2012.
23. Camilleri J, Sorrentino F, Damidot D, Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus, Academy of Dent materials, Vol: 29, Issue: 5, 2013, P: 580-593.
24. Zhenglin Yuan, Bin Peng, Han Jiang, Zhuan Bian, Ping Yan, Effect of Bioaggregate on Mineral-associated Gene Expression in Osteoblast cells, Journal of Endodontology, 36(7), 2010.
25. Yusuf-Burak Batur, Gozde Acar, Yagmur Yalcin, Seckin Dindar, Hande Sancakli, Ugur Erdemir, The cytotoxic evaluation of mineral trioxide aggregate and

bioaggregate in the subcutaneous connective tissue of rats, Medical oral Pathology Oral Cir Bucal, 18(4), 2013, 745-751.

26. Z. Oncel Torun, D. Torun, K. Demirkaya, S. T. Yavuz, M. P. Elci, M.Sarper & F. Avcu. Effects of iRoot BP and white mineral trioxide aggregate on cell viability and the expression of genes associated with mineralization. Int Endod J. 2015 Oct, Vol: 48, No: 10, P: 986-93
27. Alani AH, Toh CG. Detection of microleakage around dental restorations: A review. Oper Dent 1997, Vol: 22, P:173-85.
28. Wu MK, Kontakiotis EG, Wesselink PR. Decoloration of 1% methylene blue solution in contact with dental filling materials. J Dent 1998;26:585-9.
29. J. Danin, L. Linder. M.-L. Sund, T. Stromberg, B. Torstenson & L. Zetterqvist. Quantitative Radioactive Analysis of Microleakage Of Four Different Retrograde Fillings. International Endodontic Journal, 1992, Vol: 25, P: 183-188.
30. F, Ozata, N. Erdilek & H.Tezel. A comparative sealability study of different retrofilling materials. International Endodontic Journal 1993, Vol-26, 241-245.
31. Mahmoud Torabinejad, Robert K. Higa, Douglas J. McKendry and Thomas R. Pitt Ford. Dye Leakage of Four Root End Filling Materials: Effects of Blood Contamination. JOE, Vol. 20, No. 4, April 1994.
32. Peter A. Gilheany, David Figdor et al. Apical Dentin Permeability and microleakage associated with Root End Resection and Retrograde Filling. JOE, vol-20, No-1, January 1994.
33. Mahmoud Torabinejad, C. U. Hong, F. McDonald and T. R. Pitt Ford. Physical and Chemical Properties of a New Root-End Filling Material. Journal of Endodontics, Vol. 21, No. 7, July 1995.

34. Massimo Gagliani, Silvio Taschieri and Raffaella Molinari. Ultrasonic Root-End Preparation: Influence of Cutting Angle on the Apical Seal. JOE, vol. 24, no. 11, November 1998.

35. J. Aqrabawi et al. Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. British Dental Journal, Vol 188, No. 5, March 11, 2000.

36. Beatriz Farias Vogt, Cristina Braga Xavier, Flávio Fernando Demarco, Marcio Schüler Padilha. Dentin penetrability evaluation of three different dyes in root-end cavities filled with mineral trioxide aggregate (MTA). Braz Oral Res 2006; 20 (2):132-6.

37. Marcia Carneiro Valera, Carlos Henrique Ribeiro Camargo, Alessandra Sverberi Carvalho, Eduardo Ramalho Pereira Gama. In vitro evaluation of apical microleakage using different root-end filling materials. J Appl Oral Sci. 2006; 14 (1):49-52.

38. Letícia Kirst Post, Fábio Garcia Lima, Cristina Braga Xavier, Flávio Fernando Demarco, Marília Gerhardt-Oliveira. Sealing Ability of MTA and Amalgam in Different Root-End Preparations and Resection Bevel Angles: An In Vitro Evaluation Using Marginal Dye Leakage. Braz Dent J (2010) 21(5): 416-419.

39. Amany E. Badr et al. Marginal Adaptation and Cytotoxicity of Bone Cement Compared with Amalgam and Mineral Trioxide Aggregate as Root-end Filling Materials. JOE, Volume 36, Number 6, June 2010.

40. Shahriar Shahi, Hamid R. Yavari, Saeed Rahimi, Mahsa Eskandarinezhad, Sahar Shakouei and Mahsa Unchi. Comparison of the sealing ability of mineral trioxide aggregate and Portland cement used as root-end filling materials. Journal of Oral Science, Vol. 53, No. 4, 517-522, 2011.

41. N. Shokouhinejad, M. H. Nekoofar, H. Razmi, S. Sajadi, T. E. Davies, M. A. Saghiri, H.Gorjestani & P. M. H. Dummer. Bioactivity of EndoSequence Root Repair Material and Bioaggregate. International Endodontic Journal,Vol: 45, P1127–1134, 2012.
42. De-Deus G, Canabarro A, Alves GG, Marins JR, Linhares AB, Granjeiro JM. Cytocompatibility of the ready-to-use bioceramic putty repair cement iRoot BP Plus with primary human osteoblasts. Int Endod J 2012;45: P: 508-513.
43. Amin Salem Milani, Sahar Shakouie, Zahra Borna, Alireza Sighari Deljavan, Mohammad Asghari Jafarabadi, Fatemeh Pournaghi Azar. Evaluating the Effect of Resection on the Sealing Ability of MTA and CEM Cement. IEJ Iranian Endodontic Journal 2012; 7(3):134-138.
44. El Sayed MA, Saeed MH. In vitro comparative study of sealing ability of Diadent Bioaggregate and other root-end filling materials, Journal of conservative dentistry, 15(3), 2012, 249-252.
45. Sabari Girish C, Ponnappa KC, Girish TN, Ponappa MC. Sealing ability of mineral trioxide aggregate, calcium phosphate and polymethylmethacrylate bone cements on root ends prepared using an Erbium: Yttriumaluminium garnet laser and ultrasonics evaluated by confocal laser scanning microscopy. Journal of Conservative Dentistry, Jul-Aug 2013, Vol-16, Issue 4, 304-308.
46. Young-Eun Jang, Bin-Na Lee, Jeong-Tae Koh, Yeong-Joon Park, Nam-Eok Joo, Hoon-Sang Chang, In-Nam Hwang, Won-Mann Oh, Yun-Chan Hwang. Cytotoxicity and physical properties of tricalcium silicate-based endodontic materials. Restorative Dentistry and Endodontics, 2014, Vol: 39, No: 2, P: 89-94.
47. Pathak S. Comparative Evaluation of Sealing Ability of Root End Filling Materials: An In-Vitro Study. Int J Dent Med Res 2015; 1(5):48-52.

48. Pankaj Kumar Gupta, Gaurav Garg, Chandana Kalita, Anjan Saikia, T. S. Srinivasa, G. Satish. Evaluation of sealing ability of Biodentine as retrograde filling material by using two different manipulation methods: An in vitro study. Journal of International Oral Health, Vol: 7, Issue:7, P: 111-114.

49. Prasanti Kumari Pradhan, Sharad Kamat, Sandhya .A.K, Kanhu Charan Sahoo, Sealing Ability Of Five Different Root End Filling Materials - An Ex-Vivo Study. Journal of Dental Herald, January 2014, Issue: 1, Vol.:1.

50. Fatemeh Mokhtari, Jalil Modaresi, Gholamreza Javadi, Amin Davoudi, Hamid Badrian. Comparing the Marginal Adaptation of Cold Ceramic and Mineral Trioxide Aggregate by Means of Scanning Electron Microscope: An In vitro Study. Journal of International Oral Health, 2015,Vol: (9), P: 7-10.

51. Mayuri Mohan Naik, Ida de Noronha de Ataide, Marina Fernandes, Rajan Lambor. Assessment of apical seal obtained after irrigation of root-end cavity with MTAD followed by subsequent retrofilling with MTA and Biodentine: An in vitro study. Journal of Conservative Dentistry, Mar-Apr 2015, Vol:18, Issue: 2.

52. Pragna Mandava, Nagesh Bolla, Jayaprakash Thumu, Sayesh Vemuri, Sunil Chukka. Microleakage Evaluation Around Retrograde Filling Materials Prepared Using Conventional and Ultrasonic Techniques. Journal of Clinical and Diagnostic Research. 2015 Feb, Vol-9(2): ZC43-ZC46.

53. Ankita Khandelwal, J. Karthik, Roopa R. Nadig, Arpit Jain. Sealing ability of mineral trioxide aggregate and Biodentine as the root end filling material, using two different retro preparation techniques - An in vitro study.International Journal of Contemporary Dental and Medical Reviews (2015), Article ID 150115, 6 Pages.

54. Jun Tian, Wenting Qi, Yuanhe Zhang, Michael Glogauer, Yongqiang Wang, Zhihui Lai and Hongwei Jiang. Bioaggregate inhibits osteoclast Differentiation, fusion and Bone resorption In Vitro. JOE, Vol: 41, No: 9, September 2015.
55. Dilek Helvacioglu – Yigit, Husniye Demirturk Kocasarac, Boulos Bechara and Marcel Noujen. Evaluation and Reduction of Artifacts generated by 4 different root-end filling materials by using multiple Cone-beam Computed Tomography imaging settings. Journal of Endodontics, Vol: 42, No:2, February 2016.
56. Anurag Jain, K.C. Ponnappa, PankajYadav, Yogesh Rao, Nikhil Relhan, PriyankaGupta, Ashish Choubey, Shivanshu Bhardwaj. Comparison of the Root End Sealing Ability of Four Different Retrograde Filling Materials in Teeth with Root Apices Resected at Different Angles – An Invitro Study. Journal of Clinical and Diagnostic Research. 2016 Jan, Vol-10(1): ZC14-ZC17.
57. Harshit Srivastava, Aastha Arora Srivastava, Ashwini B Prasad and Deepak Raisingani. Sealing Ability of Glass Ionomer Cement, Biodentine, Mineral Trioxide Aggregate & Bone Cement When Used As Retrograde Filling Materials In Endodontic Surgery. International Journal of Current Medical and Pharmaceutical Research, Vol. 2, Issue, 6, pp.379-382, June 2016.
58. Raghad A. Al-Hashimi, Maha A. Habeeb, Athil A. Al-Kinani. A Comparative Evaluation of Apical Seal Associated with Ultrasonic Retrograde Cavities Filled with Bioactive Material (In Vitro Study). J Bagh College Dentistry Vol. 28(3), September 2016.
59. Teena Dsouza, Shreetha Bhandary, Raghu Srinivasan. An in vitro Comparative Evaluation of the Sealing Ability of Mineral Trioxide Aggregate with Different Mixing Reagents. World Journal of Dentistry, April-June 2016; 7(2):54-58.

60. Dr. Nalini Desai, Dr. Rajeev S, Dr. Sahana S, Dr. Jayalakshmi KB, Dr. Hemalatha B, Dr.Sivaji K, Dr. Vijay Kumar R, Dr. Vamshi Krishna V, Dr. Savitri D, Dr. Gyanendra Pratap Singh. In vitro comparative evaluation of apical microleakage with three different root-end filling materials. International Journal of Applied Dental Sciences 2016; 2(3): 29-32.
61. Wei Zhou, Qinghua Zheng, Xuelian Tan, Dongzhe Song, Lan Zhang, and Dingming Huang. Comparison of Mineral Trioxide Aggregate and iRoot BP Plus Root Repair Material as Root End Filling Materials in Endodontic Microsurgery: A Prospective Randomized Controlled Study. JOE, Volume 43, Number 1, January 2017.
62. Shubha Chhaparwal, Nidambur Vasudev Ballal, Nympha Deena Menezes, Shobha Ullas Kamath. Effect of chelating agents on sealing ability of Biodentine and mineral trioxide aggregate. Saudi Endodontic Journal, Jan-Apr 2017, Vol 7, Issue 1, 16-22.
63. Thomas von Arx et al. Apical surgery: A review of current techniques and outcome. The Saudi Dental Journal (2011) 23, 9–15.
64. Saeed Asgary, Mohammad Jafar Eghbal, Masoud Parirokh, Hassan Torabzadeh. Sealing Ability of Three Commercial Mineral Trioxide Aggregates and an Experimental Root-End Filling Material. IEJ -Volume 1, Number 3, Fall 2006.
65. Eldeniz AU, Erdemir A, Belli S. Shear bond strength of three resin based sealers to dentin with and without the smear layer. J Endod. 2005; 31:293-6.
66. Verissimo DM, do Vale MS. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review. J Oral Sc. 2006; 48:93-8.
67. Youngson CC, Jones JC, Manogue M, Smith IS. In vitro dentinal penetration by tracers used in microleakage studies. Int Endod J. 1998; 31:90-9.
68. Surgical endodontics: past, present, and future. Endodontic Topics, 2014, Vol: 30, P:29-43.

69. Kim S, Kratchman S. Modern endodontic surgery concepts and practice: A review. J Endod 2006;32:601-23.

70. Syngcuk Kim and Samuel Kratchman. Modern Endodontic Surgery concepts and practice: A review, JOE, Vol: 32, No: 7, July 2006.

71. Ivan Ivanov, Elka Radeva, Tsonko Uzunov. Endodontic Surgical treatment – A literature review, International Journal of Scientific and Research Publications, Vol:5, Issue:10, October 2015.

72. Richard Rubinstein, DDS, MS; and Mahmoud Torabinejad. Contemporary Endodontic Surgery. CDA Journal, Vol.32, No.6, June.2004.

73. Gianfranco De Paolis, Valentina Vincenti, Matteo Prencipe, Valerio Milana, Gianluca Plotino. Ultrasonics in Endodontic Surgery: A review of the literature. Annali di Stomatologia, 2010, Vol: 1(2), P: 6-10.

74. M,-K, WU & P, R, Wesselink. Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. International Endodontic Journal (1993) 26, 37-43.

75. Fernando Accorsi Orosco, Clovis Monteiro Bramante, Roberto Brandão Garcia, Norberti Bernardineli, Ivaldo Gomes De Moraes. Sealing ability, marginal adaptation and their correlation using three root-end filling materials as apical plugs. J Appl Oral Sci., 2010, Vol: 18(2), P: 127-34.

76. Eduardo Antunes Bortoluzzi, Norberto Juárez Broon, Clovis Monteiro Bramante, Roberto Brandão Garcia, Ivaldo Gomes de Moraes and Norberti Bernardineli. Sealing Ability of MTA and Radiopaque Portland Cement With or Without Calcium Chloride for Root-End Filling. Journal of Endodontics, Vol. 32, No. 9, September 2006.

77. B.S. Chong, T.R. Pitt Ford, Watson T. F. The adaptation and sealing ability of light-cured glass ionomer retrograde root fillings. International Endodontic Journal, Vol: 24, No: 5, P: 223-232, 1991.

78. Kokate R, Pawar Ajinkya. M. An in vitro comparative stereomicroscopic evaluation of marginal seal between MTA, glass ionomer cement & Biodentine as root-end filling material using 1% methylene blue as tracer. Endodontology, 2012, Vol: 1, P:36-42.

79. Sawsan Taha Hassan Abu Zeid, Osama S. Alothmani, Mohammed Khalil Yousef. Biodentine and Mineral trioxide Agreggate: An analysis of solubility, pH changes and leaching elements. Life Science Journal, 2015, Vol: 12, No: 4, P: 18-23.

80. Storm B, Eichmiller FC, Tordik PA, Goodell GG. Setting expansion of gray and white mineral trioxide aggregate and Portland cement. J Endod 2008;34(1):80-2.

81. Ravichandra PV, Vemisetty H, Deepthi K, Reddy SJ, Ramkiran D, Krishna MJ et al. Comparative evaluation of marginal adaptation of Biodentine (TM) and other commonly used root end filling materials – An in vitro study. J Clin Diagn Res. 2014; 8:243-5.

INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN
SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST
Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945.

No: VDCW/IEC/06/2015 Date: 14.12.2015

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. Vishnuvardhini.S.

Title: Assessment of Apical Sealing Ability of Retrograde Filling Materials with GIC,MTA,Biodentine and Bioaggregate: An invitro study.

Institutional ethics committee thank you for your submission for approval of above proposal .It has been taken for discussion in the meeting held on 04 .12.15.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205
Tiruchengode (Tk) Namakkal (Dt),
Tamilnadu.

SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205.
Tiruchengode (Tk) Namakkal (Dt),
Tamilnadu.